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FOOD INVESTIGATION

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RANCIDITY IN EDIBLE FATS

BY

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PREFATORY NOTE

THE object in compiling this report has been to present an account of the current state of knowledge concerning the development of rancidity in edible fats and fat-containing foods. Emphasis has been laid chiefly on the more scientific aspects of the problem, but details of methods likely to prove of practical value in the diagnosis and correction of faults have also been included.

It has not been possible to answer satisfactorily many of the questions raised, but research in this branch of chemistry is at present extremely active, and it is probable that further progress in the elucidation and control of the changes involved will be rapid.

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RANCIDITY IN EDIBLE FATS

PART I.—THE CHEMISTRY OF THE FATS

Importance as Food

PROTEIN, carbohydrate and fat, together with water, constitute a very large proportion of man's food. In Table 1 are given data for the fat-content of a few of the commoner foodstuffs; the figures quoted can serve only as a rough guide, since in many cases the variation from sample to sample is great.

TABLE 1.—*Approximate fat-content of some common foodstuffs.*

Food.	Fat (per cent.)		Food.	Fat (per cent.)	
	On total weight.	On dry weight.		On total weight.	On dry weight.
Salad-oils, lard, pastry shortenings, cooking fats, cod-liver oil	100	100	Cod and haddock	0·4	2
			Trout	2	10
			Halibut ..	5	20
			Mackerel ..	7	26
			Salmon	13	35
Butter	85	95	Pilchard ..	14	40
Margarine ..	86	98	Herring ..	1–25	5–50
Cheese	20–44*	30–67			
Milk	4	30	White bread ..	1·1	1·9
Cream	20–60	75–95	Brown bread ..	1·5	2·6
Eggs (whole) ..	11	40	Oatmeal ..	8	9
Eggs (yolk) ..	33	63	Sponge-cake ..	11	13
Eggs (white) ..	0·2	1·2			
			Vegetables ..	0·1–0·5	1–5
Beef-steak ..	19	49	Fruits	0·2–1·5	1–8
Lamb chop ..	28	60			
Pork chop ..	30	63	Honey	0	0
Bacon	37–61	72–85	Sugar	0	0

Protein is required chiefly for growth, and for the repair and renewal of worn-out tissues. Carbohydrate and fat provide the energy by which the temperature of the body is maintained and the activities of life carried out. Naturally, so simple a picture only approximates to the truth. Protein consumed in excess of requirements is, in fact, burned for fuel, and carbohydrate and fat are built into the body's structure. Mineral salts, including traces of many elements, and the accessory food-factors or vitamins, are also essential to life. For the provision of energy, fat is the ideal food. Approximate amounts of energy liberated when 1 gram of the substance is completely oxidised in the body are 9·3, 4·1 and 4·2 Calories respectively for fat, protein and carbohydrate. Fat is therefore the most concentrated form in which the organism can store

* Skimmed-milk cheeses 7–25 per cent., cream cheeses 40–90 per cent. fat.

energy; hence the presence of fat round the embryo in the seeds of plants and in the yolk of the egg, and in the adipose tissues of animals. The deposits of fat which warm-blooded animals lay down immediately beneath the skin probably also serve a useful purpose in assisting to conserve the bodily heat.

In addition to being burned for energy, fat enters to some extent into the composition of basic protoplasm, usually as compounds more complex than the simple glycerides of the stored fats. It has even been claimed that some animals, at least, cannot synthesise certain of the unsaturated fatty acids (e.g. linoleic), and that symptoms of deficiency, disease and ultimately death ensue if the food does not contain these acids.[7, 21]*

Occurrence in Vegetable and Animal Tissues

As already stated, fats or (to use the more general term) lipoids occur in the organism both as universal constituents of protoplasm and of cell-membranes, and in localised deposits as a reserve supply of food. In the cell the particles of fat may range in size from invisible colloidal particles to droplets easily visible under the microscope. In tissues specially adapted for the storage of fat, the cell is practically composed of one large droplet of fat enclosed by the cell-membrane. Tissues of this kind can contain up to 96 per cent. of fat.

In the vegetable kingdom fats are synthesised from carbon dioxide and water under the influence of sunlight, probably *via* the intermediate formation of carbohydrate. Stored fat is deposited mainly in the seed, and sometimes also in the fleshy pericarp or fruit which encloses it; these are the sources of the vegetable fats of commerce. Fat rarely occurs in considerable quantity in the roots, stems and leaves of plants.

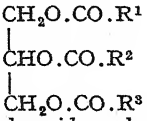
The main deposits of fat in terrestrial animals are usually immediately beneath the skin, and surrounding certain organs, such as the kidneys, stomach and intestine. Considerable amounts are frequently also present in organs, particularly the liver, in the bone-marrow, and between and within the muscles. In case of fish, the location of the main reserve of fat varies with the species. "Fatty" fish, such as the herring, sardine, mackerel and salmon, deposit the major part of their oil in the depot beneath the skin, between the muscles, and around various organs. Others, including the cod, ling and haddock, deposit large quantities of oil in the liver, which in these species is abnormally large and fatty, while the muscle contains relatively little fat. In both types there is a pronounced seasonal variation in the reserves of fat, depending on the food-supply and on the reproductive cycle. During spawning the fish practically cease to feed, and the reserves of fat, which had reached a high level, almost disappear. Fat-contents of the

* The figures in brackets refer to the List of References at the end of each Part.

flesh of the herring, for example, have been found as high as 25 per cent. and as low as 1 per cent.[52] Marine mammals (whales, seals, etc.) deposit great thicknesses of blubber beneath the skin. Deposits of fat in unusual places are those of the cavities of the head and jaw in the sperm-whale and porpoise.

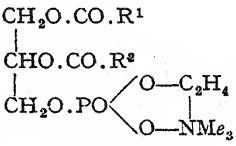
Types of Natural Fatty Compounds

The acids which form the units from which individual fats are built up exist in nature combined in a variety of ways. The ordinary fats consist of complex mixtures of glycerides, compounds in which the trihydric alcohol, glycerol, is esterified with three molecules of the same or of different fatty acids.

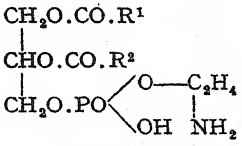


A triglyceride molecule

In addition to these there are the phosphatides—lecithins, kephalins and related substances of great physiological importance, which contain, in addition to glycerol and fatty acids, a molecule each of phosphoric acid and of a nitrogenous base. Lecithin and kephalin, which contain the bases choline and amino ethyl alcohol, have the formulæ*—



Lecithin



Kephalin

Other substances—the cerebrosides—are compounds of fatty acids with a carbohydrate and a nitrogenous base. The waxes, which are esters of the fatty acids with the higher fatty alcohols, are widely distributed in small amounts in the vegetable kingdom, and occur in larger quantity in spermhead oil and in the various insects' waxes. Quite recently substances isolated from the liver-oils of certain fish and termed chimyl, batyl and selachyl alcohols, have been shown to be ether-alcohols formed by condensation of glycerol with a fatty alcohol. In the natural oils they probably occur in combination with fatty acids.

In considering the problem of rancidity in foods the main concern will be with changes in the simple triglycerides of the fats proper, changes in more complicated fatty compounds being only briefly touched upon.

The Fatty Acids

At least 40 different fatty acids are known. The number in an individual fat is never less than three or four, is often seven or

* The formulæ given represent the α-compounds. Lecithins and kephalins in which the phosphoric acid is attached to glycerol in the β-position also occur.

eight and may be 15 or more. The acids themselves are straight-chain aliphatic monocarboxylic compounds containing from four to 30 or more carbon atoms, and from none to five or six double bonds. Almost invariably the acids contain an even number of carbon atoms. Hydroxy acids, cyclic acids, acids containing a triple linkage, and acids containing an odd number of carbon atoms, are found in a few fats, but are exceptional and of rare occurrence.

TABLE 2.—*Physical properties of the common saturated fatty acids.**

Acid.		Melting point. (°C.)	Boiling point. (°C.)	Solubility in water		Volatility in steam.	Odour.
				15°C.	100°C.		
Butyric	C4	−8	163	Miscible		Volatile	Rancid
Caproic	C6	−1·5	205	0·88%	—	,,	,,
Caprylic	C8	16	237	0·08%	0·25%	,,	,,
Capric	C10	31·3	269	—	0·1%	,,	Weak
Lauric	C12	43·5	102/1 mm.	—	Very slight	Slight	Odourless
Myristic	C14	53·8	122/1 mm.	Insoluble		Very slight	,,
Palmitic	C16	62·5	139/1 mm.	,,		Non-volatile	,,
Stearic	C18	69·6	160/1 mm.	,,		,,	,,
Arachidic	C20	77	205/1 mm.	,,		,,	,,
Behenic	C22	82	—	,,		,,	,,
Lignoceric	C24	86	—	,,		,,	,,

* Melting and boiling points according to Hilditch in Schönfeld's *Chemie und Technologie der Fette und Fettprodukte*, Vol. I, Vienna, 1936.

The saturated acids (Table 2) have the general formula $C_nH_{2n}O_2$. The lower members of the series are liquid, volatile and steam-volatile, soluble in water and possessed of a strong odour. The higher members are solid, non-volatile, insoluble, odourless and tasteless. Palmitic, followed by stearic, myristic and lauric, is the most widely distributed of the saturated acids.

The commonest unsaturated acids belong to the C_{18} series, oleic being by far the most abundant constituent of natural fats.

Oleic acid .. $CH_3.(CH_2)_7.CH = CH.(CH_2)_7.COOH$
Linoleic acid .. $CH_3.(CH_2)_4.CH = CH.CH_2.CH = CH.(CH_2)_7.COOH$
Linolenic acid .. $CH_3.CH_2.CH = CH.CH_2.CH = CH.CH_2.CH = CH.(CH_2)_7.COOH$

The unsaturated acids are liquid at ordinary temperatures, as also in general are glycerides containing two or more molecules of unsaturated acid. Whether a fat is solid or liquid therefore depends largely* on the relative proportions of saturated and unsaturated acids it contains. Isomers of oleic acid in which the double bond is in other than the 9 : 10 position, i.e. vaccenic ($\Delta^{11:12}$) and petroselinic ($\Delta^{6:7}$) acids, and monoethylenic acids containing 10, 12, 14,

* Some unsaturated acids, e.g., erucic, petroselinic and iso-oleic, are solid. Glyceride structure has also a considerable influence on consistency.

16, 20, 22 and 24 carbon atoms, are also known. Other linoleic and linolenic acids exist, but those present as major constituents of the common fats correspond to the formulæ given. Elæostearic acid of China-wood oil is an isomer of linolenic acid which has three conjugated double bonds, $CH_3.(CH_2)_3.CH=CH.CH=CH.CH=CH.(CH_2)_7.COOH$. Still more highly unsaturated acids, e.g., arachidonic (C_{20} with four double bonds), occur in small quantities in fat from the liver and other organs of mammals and birds, in traces in the depot fats, and particularly as constituents of the phosphatides of brain, liver, and egg-yolk. The oils of fish and other marine animals contain a whole series of unsaturated acids with from 18 to 24 carbon atoms and from four to six double bonds. Formulæ have been assigned to a number of these acids, but the positions of the double bonds in most cases still cannot be regarded as settled.

The Fatty-acid Composition of Fats

The tissues of all plants and animals contain fat, which frequently varies considerably in composition, according to the part of the organism from which it is extracted. The number of the natural fats is therefore very large. Grün and Halden[26] have listed and characterised some 1,400 distinct fats, few of which have attained commercial importance.

Elucidation of the fatty-acid composition of a fat is a straightforward, though somewhat lengthy, procedure. The saponification- and iodine-values of the mixed fatty acids give the mean molecular weight and total unsaturation, but these alone provide little information as to the nature and proportions of the individual acids present. An approximate estimate of the composition of simpler mixtures can be obtained by determination also of the thiocyanogen-value*, or of the thiocyanogen-value and the percentage of saturated acids present (by Bertram's or Twitchell's method), but for complete analysis of the constituent acids of a fat fractionation of the methyl esters is essential. The method employed in this case is as follows.

The fat is extracted from the tissue, saponified with alcoholic potash, and the free acids recovered. These are then treated with lead acetate in hot alcoholic solution and thereby separated into two fractions, (*a*) the "solid acids," consisting of saturated acids, together with a little oleic but none of the more highly unsaturated acids; and (*b*) the "liquid acids," which contain the remainder of the unsaturated acids, together with traces of palmitic and myristic, and a considerable proportion of the saturated acids of still lower molecular weight when these are present. The two groups of acids are then converted into methyl esters by refluxing with methyl alcohol and 2–3 per cent. of sulphuric acid, and

* Thiocyanogen reacts with the single double bond of oleic, with one of the two double bonds of linoleic and with two of the three double bonds of linolenic acid. Iodine reacts with them all.

fractionally distilled from a Willstatter flask at a pressure of not more than 1 mm. of mercury. Unless the mixture happens to be a very simple one, it will be necessary to redistil one or more of these fractions. In this way fractions of " solid " esters are obtained which consist largely of two saturated acids and oleic, and can therefore be analysed from saponification- and iodine-values alone. In distilling the " liquid " esters, the esters of the saturated acids of low molecular weight come over in the earlier fractions. If any of these mixed fractions are too complex to be analysed by saponification- and iodine-values alone, the unsaturated esters can be destroyed by oxidation with permanganate, and the saturated acids recovered and examined separately. The simpler unsaturated acids are identified by conversion into crystalline polyhydroxy derivatives by treatment with permanganate in ice-cold alkaline solution. The constitution of a less usual or more complex unsaturated acid is established by oxidising its ester with powdered potassium permanganate in acetone solution, or by treatment with ozone. In either case the carbon chain is broken at the double bonds and the fragments identified.

The fatty-acid compositions of a considerable number of vegetable and animal fats have been investigated in this way by workers in various countries, and particularly by Hilditch and his collaborators at Liverpool. The data show that the composition of the fat is closely allied to the biological species, organisms of related species producing fats of similar general type. Palmitic and oleic acids seem invariably to be present, and very many fats consist of these, accompanied only by minor proportions of stearic, myristic or linoleic acids. The majority of the fats of seeds are of this type (Table 3). On the other hand, in certain botanical species a fatty acid or acids in addition to those already mentioned appears in quantity in the seed-fat, and in such cases the occurrence of this acid is almost wholly confined to one, or at most to a few families of plants. As cases in point may be quoted erucic acid, $C_{22}H_{42}O_2$, which is present in quantity up to 40–50 per cent. in all the *Cruciferæ*; petroselinic acid, $C_{18}H_{34}O_2$ ($\triangle^{6:7}$), similarly present in the *Umbelliferæ*; and arachidic, $C_{20}H_{40}O_2$, and lignoceric, $C_{24}H_{48}O_2$, acids in the *Sapindaceæ* and some of the *Leguminosæ*. Stearic acid is only present in large amount in the seed-fats of a few tropical families, and the same is true of myristic and lauric acids. The kernel-fats of the *Palmæ* are remarkable for the presence of large amounts of lauric acid and of appreciable quantities of caprylic, $C_8H_{16}O_2$, and capric, $C_{10}H_{20}O_2$ acids.

Complete analyses are as yet available for the fats of only a very few terrestrial animals and birds (Table 4). So far as can be judged from these data, the chief constituents of the fats are palmitic, oleic and linoleic acids, supplemented in some of the herbivora by comparatively large amounts of stearic, and in a few cases by appreciable quantities of palmitoleic, $C_{16}H_{30}O_2$, or myristic

TABLE 3.—*The component fatty acids of some vegetable fats.**

Fat.	Approximate iodine-value.	Fatty Acids (%)						
		Myristic.	Palmitic.	Stearic.	Arachidic	Oleic.	Linoleic.	Linolenic.
Palm-oil	53–58	2	40	4	—	44	10	—
Olive-oil	79–86	—	10	2	—	81	7	—
Avocado-pear oil	85–88	—	7	1	—	81	11	—
Oat (germ) oil	100–110	—	10	—	—	59	31	—
Barley (seed) oil	110–130	—	9	3	—	33	54	1
Rice (meal) oil	95–110	—	13	2	—	44	39	—
Rice (bran) oil		—	18	3	—	48	29	—
Rye (germ) oil	110–130	—	9	—	—	35	48	5
Wheat (germ) oil	110–130	—	14	1	—	30	44	11
Maize (germ) oil	110–130	—	8	4	—	46	42	—
Cottonseed oil	104–113	1	21	2	—	30	46	—
Soya-bean oil	125–140	—	8	5	—	28	54	4
Linseed oil	170–185	—	5	4	—	10	43	38
Sesame-oil	103–117	—	8	5	—	49	38	—
Arachis (peanut) oil	85–95	—	8	4	6	61	21	—
Cacao-butter	35–42	—	24	35	—	39	2	—
Shea-butter	56–67	—	9	36	—	50	5	—
Coconut-oil†	8–10	18	10	2	—	7	2	—
Palm-kernel oil†	15–20	14	9	1	—	18	1	—

* The figures in Tables 4, 5 and 6 (which, in a number of cases, are the average of several analyses) are given to the nearest whole number, figures of 0·5 per cent. and below not being recorded. The majority of the data are reproduced from papers by Hilditch and his collaborators.

† Coconut-oil contains also 8 per cent. caprylic, 7 per cent. capric and 46 per cent. lauric acid, and palm-kernel oil 3 per cent. caprylic, 7 per cent. capric and 47 per cent. lauric acid.

TABLE 4.—*The component fatty acids of some terrestrial animal fats.**

Fat.	Fatty acids (%)							
	Myristic.	Palmitic.	Stearic.	Palmitoleic.	Oleic.	Linoleic.	Linolenic.	Highly unsaturated acids.
Hen (abdominal)	—	26	7	7	38	21	—	trace
,, (gizzard)	—	25	7	8	37	23	—	,,
,, (neck)	—	27	6	7	39	21	—	,,
,, (egg-yolk)	31			52		15	2	?
Goose	—	21	11	—	49	19	trace	trace
Emu (perinephric)	40			—	49	11	—	?
Pig (outer back)	2	24	11	—	53	10	—	trace
,, (inner back)	1	30	14	—	47	8	—	,,
,, (leaf)	4	29	21	—	41	5	—	,,
,, (composite)	1	28	9	—	57	5	—	,,
Sow (outer back)	4	20	8	—	54	13	—	1
,, (inner back)	4	26	11	—	44	14	—	1
,, (leaf)	4	28	17	—	36	14	—	1
Beef Tallow	3	25	24	2	42	2	—	0·5
,, ,, ...	4	31	19	—	43	3	—	?
Mutton tallow	2	27	31	3	32	4	—	?
Rabbit (perinephric)	5	23	4	—	3	56	9	?
Rat (body)	5	25	2	8	58	2	—	—
Horse (body)	—	27	5	—	50	13	5	?
Reindeer (loin)	7	35	20	—	37	—	—	?

* Recent work indicates that the depot fats of the pig, ox and probably of other animals contain small amounts of palmitoleic and traces of myristoleic acid. Larger amounts (5–10 per cent.) of these acids and of the highly unsaturated C_{20-22} acids (5–25 per cent.) are present in the glycerides and phosphatides of the liver.

acid. Traces of highly unsaturated C_{20} and C_{22} acids have been detected in several body- and milk-fats, and are present in much greater quantities in the phosphatides of liver, brain, egg-yolk, etc. The milk-fats of the larger land mammals appear to be unique in containing small but important amounts of butyric, caproic, caprylic and capric acids (Table 16). The influence of factors such as age, diet and body-temperature on the composition of animal fats will be considered in a later section.

TABLE 5.—*The component acids of the fats of some aquatic organisms.*

Source.	Fatty acids (%)							
	Saturated.			Unsaturated.*				
	C_{14}	C_{16}	C_{18}	C_{14}	C_{16}	C_{18}	C_{20}	C_{22}
Marine.								
Haddock (liver)	5	15	—	1	14	31(2·6)	27(5·9)	7(7·3)
Whale(blubber)	6	11	3	1	17	40(2·5)	10(7·2)	12(8·3)
Cod (liver) ..	5	8	—	—	17	28(3·0)	29(5·7)	11(7·0)
Halibut (liver)	5	16	1	—	20	34	13(5·5)	11(7·6)
Herring (body)	7	14	2	—	16	27(3·3)	21(5·9)	13(8·6)
Copepod ..	10	12	1	2	13(2·4)	17(5·1)	23(7·8)	22(8·1)
Zooplankton..	4	14	2	3(?2)	22(·2)	43(5·0)	11(8·0)	—
Freshwater.								
Carp (body) ..	4	16	2	1	19	44(3·2)	14(6·9)	—
Pike (body) ..	6	14	1	1	22	37(3·0)	14(7·5)	5(7·5)
Perch (body) ..	4	13	2	1	21	40(3·2)	13(6·8)	6(9·2)
Copepod ..	7	16	1	3(2·7)	32(3·0)	24(5·2)	14(8·6)	3(?)

* The unsaturated acids have only been divided into groups according to molecular weight, the mixtures being too complex for resolution into individual acids. The figures in parentheses indicate *the average unsaturation of the fraction*, the absence of a figure in the case of an unsaturated acid denoting an unsaturation of 2·0, i.e. one double bond.

Fats from marine sources are sharply differentiated from those of terrestrial origin by being largely composed of unsaturated acids containing from 14 to 24 carbon atoms and from one to six double bonds (Table 5). This basic type of fat appears to be general for all life in the sea, from the minute flora and fauna of the plankton to the largest fish and marine mammals. Some modification, however, occurs in certain families, as for example in the liver-oils of some elasmobranch fish, in which the full range of fatty acids is present, but their unsaturation is practically restricted to one double bond. In these cases comparatively large amounts of the highly unsaturated hydrocarbon squalene or of batyl and selachyl alcohols frequently accompany the glycerides. Freshwater plants and animals differ

slightly from marine in showing an increased content of palmitoleic and C_{18} acids, and a corresponding decrease in C_{20} and C_{22} acids. The body-fat of the salmon, a fish which changes its habitat from fresh to salt water, progresses during development from a purely freshwater to a marine type[46].

The depot fats of the few reptiles and amphibians thus far examined appear to be intermediate in composition between the fats of terrestrial and aquatic animals, a finding of considerable biological interest.*

The Glyceride Structure of Fats

Only within the last few years has appreciable progress been made in elucidation of the manner in which the constituent fatty acids are combined together as glycerides in fats. It is still not possible to obtain data on glyceride structure approaching in accuracy the fatty-acid analyses, but much general information has been collected, and in a few of the simpler cases more detailed results are now becoming available.

Even in a fat containing only three fatty acids, no less than 18 different triglycerides are theoretically possible, and this number rises very rapidly as the number of acids present increases. The glyceride molecules are so large and differ so slightly in molecular weight as to render direct separation by physical means extremely laborious. Nevertheless, sufficient qualitative data have been obtained by fractional crystallisation definitely to dispose of the original belief that fats are composed of mixtures of simple triglycerides such as tristearin and tripalmitin, since these, if present, should have been the first to be isolated, whereas, in fact, mixed glycerides were nearly always obtained. In recent work more efficient methods combining both chemical and physical processes have been evolved. These include :

(*a*) Fractional crystallisation of the fully hydrogenated fat, by which means in suitable cases an approximate estimate of the total tri-C_{18}-glycerides present in the original fat can be obtained[32].

(*b*) Fractional crystallisation of the solid addition-products obtained by bromination of the fat. Numerous crystalline brominated glycerides have been isolated in this way from highly unsaturated fats of the linseed-oil and fish-oil types, chiefly by Suzuki in Japan and by Eibner in Germany. The method is only qualitative and cannot readily be applied to the non-drying oils and solid fats.

(*c*) Quantitative separation of the fully saturated glycerides present in a fat[33]. The fat is oxidised in boiling acetone solution with potassium permanganate, whereby all the unsaturated fatty-acid chains are broken at the double bonds. Glycerides containing

* For a general survey of the component acids of natural fats see HILDITCH T. P., *Chem. Ind. Rev.*, 1935, **54**, 139, 163, 184, and HILDITCH, T. P. and LOVERN, J. A., *Nature*, 1936, **137**, 478.

one, two or three molecules of unsaturated acid are converted into compounds containing one, two or three carboxyl groups, and subsequently removed by treatment with alkali from the fully saturated glycerides which have not been attacked. From the percentage of fully saturated glycerides and the fatty-acid composition of the fat, the limiting amounts of the three other types of glyceride (mono-, di- and tri-unsaturated) present can be calculated, and in a few favourable cases a considerable proportion of the mono-acidic oxidation product (the mono-unsaturated glycerides of the original fat) has been isolated. Further information concerning the glyceride structure, especially of the less saturated fats, can be obtained by examination of the fully saturated glycerides present after hydrogenation to various stages short of complete saturation[37].

A summary of the results obtained to date shows that in most natural fats the glyceride composition tends toward that in which the fatty acids are distributed as evenly as possible in combination throughout all the triglyceride molecules. This has been shown particularly clearly for the very large number of seed-fats examined, a series ranging from liquid oils containing as little as 10 per cent. of saturated acids to solid fats with over 90 per cent. In no case does any appreciable quantity of a simple triglyceride appear until one acid is present in very large excess over the others. Similarly, no fully saturated (or fully unsaturated) glyceride is found unless saturated (or unsaturated) acids form more than 60 per cent. of the total. In some cases at least, however, there does seem to be a selective influence at work, determining the configuration of the glycerides formed. Thus, the mono-oleo-disaturated glycerides present in large amount in some of the solid seed-fats appear to exist predominantly in one form, e.g., β-palmito-oleostearin and β-oleodistearin present in cacao-butter to the extent of about 50 per cent. and 20 per cent. respectively, and β-oleodistearin present to about 65 per cent. in *Allanblackia* and *Palaquium* fats.

The oils of marine animals apparently consist of a complex mixture of glycerides in which the large number of acids present are linked together, two and three at a time, in numerous combinations. Here also simple triglycerides seem to be the exception rather than the rule.

In many of the depot fats of terrestrial animals in which stearic acid is only a minor component, e.g., those of rodents and birds, fully saturated glycerides are present only in very small amount, and the fats are constructed on the same "evenly distributed" plan which is found in most of the vegetable fats. This generalisation does not hold, however, for the milk-fats and for the body-fats of the ox, sheep and pig. In all of these cases the amount of fully saturated glycerides present is very much greater for a given ratio of saturated to unsaturated acids in the whole fat than would be anticipated from the "even distribution" law. Thus, mutton-tallow and cacao-butter both contain approximately 60 per cent. of

saturated and 40 per cent. of unsaturated acids, yet the former has about 26 per cent. and the latter only 2 per cent. of fully saturated glycerides. Butter-fat has approximately 30-40 per cent., beef-fat about 26 per cent. and lard from 2 to 20 per cent. of fully saturated glycerides. Naturally, these values depend upon the degree of unsaturation of the fat, which is liable to vary considerably with diet, etc. In order to account for such departures from the " even distribution " rule it has been suggested[3] that the stearoglycerides of the fats in question may have been produced by hydrogenation of preformed, evenly distributed oleo-glycerides.

The importance of glyceride structure in determining the properties of a fat can be brought out very clearly by reference again to cacao-butter and mutton-tallow. The component acids of these two fats are practically identical. Cacao-butter, being built up on the " even distribution " plan, contains only traces of fully saturated glycerides and probably no (liquid) triolein. It is very largely composed of mono-oleins (mainly oleopalmitostearin) which are solids, melting at about 40° C., with smaller amounts of dioleins[42]. The result is a fat of very homogeneous texture, possessing the non-greasy feel, brittleness or snap, and comparatively low (30-34° C.) sharp melting point which makes it the ideal fat for chocolate. A very recent (1936) analysis of cacao-butter by methods (*a*) and (*c*)[39] is as follows :—

		Per cent.
Fully saturated glycerides	Palmitodistearin and Stearodipalmitin	2
Mono-oleins	Oleopalmitostearin (mainly β–palmito–)	52
	Oleodistearin (mainly β–oleo-) ..	19
	Oleodipalmitin (mainly β–oleo–) ..	6
Di-oleins	Stearodiolein	12
	Palmitodiolein	9
Tri-olein	.. ..	—

Mutton-tallow on the other hand is a very heterogeneous mixture, consisting of about 26 per cent. of hard, high-melting, fully saturated glycerides, about 30 per cent. of mono-oleins and over 40 per cent. of liquid di-oleins[33]. The result is a fat which is oily or greasy at ordinary temperatures, and yet does not melt completely below about 45°-50° C.

The fats of the pig and of the hen have also been examined by these methods[37, 38]. The pig's fat contained 15 per cent. of tri-C_{18}-glycerides and nearly 80 per cent. of a mixture of monopalmito-diunsaturated glycerides with palmitostearo-oleins. Much if not all of this palmitic acid was attached to the β-hydroxyl groups of the glycerol molecules. The hen's fat contained about 30 per cent. each of tri-C_{18} and of dipalmito-C_{18}-glycerides, with only 40 per cent. of monopalmito-glycerides.

Digestibility

The characteristic aromas and flavours of the various fats are due mainly to the presence of traces of non-fatty materials, pure,

fresh glycerides of the higher fatty acids being odourless and tasteless. Ease of assimilation, on the other hand, depends to some extent on melting point, liquid oils and the softer solid fats being almost completely digested,[48] while fats which have been hydrogenated to a melting point of 50° C. or above are utilised less efficiently*. Pure tripalmitin (m.p., 63° C.) and tristearin (m.p.,71° C.) are very little absorbed, whereas liquid triolein is readily assimilated[53]. Increasing dispersibility and solubility of the fatty acids with increasing unsaturation probably also contribute to preferential absorption of the unsaturated acids in the intestine.

The complex mixture of glycerides constituting a natural fat may be fluid or largely fluid at 37° C., even though it contains appreciable quantities of palmitostearins, melting in the pure state at 63°-67° C. The harder tallows, however, which include up to 25 per cent. of these high-melting glycerides, are appreciably less readily assimilated than the lower-melting fats and oils[31]. Differences in melting point are largely determined by the proportions of the higher saturated and unsaturated acids present, but in fats containing considerable amounts of saturated acids, glyceride structure also plays a part. This has already been brought out clearly in the comparison drawn between cacao-butter and mutton-tallow.

The Unsaponifiable Constituents of Fats

Associated with fats in the tissues, and still present in fat which has been separated by pressing, rendering or extracting with solvents, are small quantities of non-fatty substances, usually classed together as "unsaponifiable matter." These substances, being insoluble in water and alkalies but soluble in fat-solvents, can be separated from the fat, after saponification with alkali, by extraction with ether or petroleum ether. Coconut, palm-kernel and soya-bean oils, and beef, mutton and butter fats, contain 0·2-0·4 per cent. of unsaponifiable matter; palm, arachis, linseed and cod-liver oils up to 1·per cent.; olive, cottonseed and sesame oils up to 1·5 per cent.; and maize, wheat, whale and herring oils up to 4 per cent. Cases in which a large proportion (30-50 per cent.) of the fat consists of unsaponifiable material are the head and blubber oils of the sperm-whale, and wool-grease, where higher fatty alcohols to a considerable extent replace glycerol in combination with the fatty acids. These alcohols, being insoluble in water, appear in the estimation as "unsaponifiable matter." Insects' waxes, such as beeswax, are largely composed of fatty alcohol-fatty acid esters. The liver-oils of certain fish, e.g., sharks, rays and dog-fish, also contain large amounts of non-fatty substances, consisting in this case mainly of the unsaturated terpene hydrocarbon squalene, $C_{30}H_{50}$, or of chimyl, batyl and selachyl alcohols.

*Differences in digestibility of oils and of fats melting below about 43°C. are, however, insignificant, assimilation being 95-98 per cent. efficient

The unsaponifiable substances considered below exist only as minor constituents in common fats. They are, however, of considerable importance, owing to their influence on appearance, nutritive value and keeping properties.

Sterols

The sterols are cyclic unsaturated alcohols of high molecular weight which occur to some extent in all fats, and frequently constitute a large proportion of the unsaponifiable fraction. Cholesterol, $C_{27}H_{45}OH$, the most abundant of the animal or zoosterols, is present mainly as ester in combination with fatty acids in wool-fat and in liver-oils, and in smaller quantity in the depot fats of all terrestrial and marine animals. Fats of vegetable origin contain the closely related phytosterols which, though very similar in general properties to cholesterol, are nevertheless sufficiently different in melting point and crystalline form to enable animal and vegetable fats to be distinguished by this means. Ergosterol, $C_{28}H_{43}OH$, the chief sterol of the fungi, on irradiation with ultraviolet light undergoes isomeric change and is converted into calciferol, which has powerful vitamin D activity. Until recently it was believed that this substance was identical with the natural vitamin, and was the only substance possessing antirachitic properties. The presence of ergosterol had therefore to be assumed in all materials which acquired activity on irradiation. Recent biological and chemical work has shown this view to be untenable. The natural vitamin D of tuna-liver oil, for example, is *not* identical with calciferol, but is probably the corresponding irradiation-product of the related 7-dehydro-cholesterol.* Calciferol, which apparently possesses the same order of activity as the cod-liver oil or irradiated-milk vitamin for human beings and for rats, is *very* much less effective for the prevention and cure of rickets in chickens. At least five different sterols possessing various degrees of activity have now been obtained in the laboratory, and it is probable that several forms of the vitamin occur in nature.

Vitamins

Of the better known vitamins, A, D and E are fat-soluble and occur in association with fats. Substances possessing vitamin D activity, as already mentioned, can be produced artificially by irradiation of ergosterol or certain other sterols, or of materials containing them, as by irradiation of foods, such as milk or yeast, or by exposure of the skin itself to sunlight. Vitamin D also exists preformed in the liver-oils of certain fish, such as the cod and halibut, in smaller quantity in egg-oil, and in still smaller quantity in milk. Refined vegetable oils in general tend to be deficient in vitamin D, but many of them acquire antirachitic activity on irradiation. Margarines made from vegetable fats are now frequently enriched by addition of vitamin concentrates. The quantity of the vitamins

* There is now, however, some evidence that more than one antirachitic substance is present in fish-oils.

present in natural fats is exceedingly small ; about 40 tons of butter or several hundredweights of cod-liver oil, for example, would be necessary to provide the equivalent of 1 gram of pure vitamin D.

Vitamin A, the growth-promoting, anti-ophthalmic vitamin, is derived by animals from the carotinoids or from preformed vitamin of their food and stored in the tissues, particularly in the liver. The liver-oils of fish and of animals such as the sheep, ox and pig are therefore the richest natural sources of vitamin A, which is also present in egg-oil, butter-fat and beef and other animal fats. The actual potency of these substances is very variable. In terms of cod-liver oil as unity, approximate estimates may be given for butter-fat and ox or halibut liver-oil as 0·02 and 100. Green vegetables and other carotene-rich materials, such as carrots and palm-oil, also appear to be active, since animals which consume them are able to utilise carotene for the production of vitamin A. The source of the large amounts of vitamins A and D in the liver-oils of certain fish is of interest. Vitamin A is undoubtedly derived from carotinoids originally synthesised by the minute green plants of the plankton, which in turn form the food of the small crustacea and fish on which larger fish like the cod and halibut feed. The origin of the vitamin D, though probably also the food of the fish, is more open to doubt, since no particularly rich source of the vitamin has been found in the food.

Vitamin E, an accessory food-factor necessary for reproductive fertility in animals, occurs most abundantly in the germ-oils of cereals. It is also present in other vegetable oils, such as cottonseed and hydrogenated cottonseed oils, and in green vegetables.

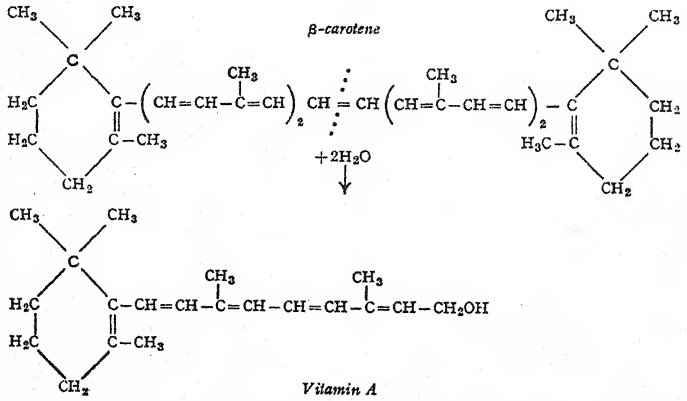
Lipochromes

The lipochromes or carotinoids are a group of highly coloured substances which constitute the yellow and red pigments of the fats and of many plant and animal tissues. They are soluble in fats and fat-solvents, insoluble in water and stable towards alkalies, but more or less susceptible to oxidation and bleaching by atmospheric oxygen. They give characteristic colour reactions with antimony trichloride, and contain only carbon and hydrogen or carbon, hydrogen and oxygen.

The carotenes and lycopene are isomeric hydrocarbons of formula $C_{40}H_{56}$, which owe their intense colour to the presence in the molecule of a system of from 11 to 13 conjugated double-bonds. In addition, there are xanthophyll (lutein) and zeaxanthin, which are dihydroxy carotenes of formula $C_{40}H_{54}(OH)_2$, and a number of other oxy-derivatives of lesser importance. These substances are widely distributed in plant tissues and enter the animal organism *via* the food, the higher animals being apparently unable to synthesise them. Of the three carotenes, the α- and β-forms frequently occur together, the β-form invariably predominating and sometimes (as in

grass) being practically the only one present. γ-carotene rarely constitutes more than a minute fraction of the total pigment. Lycopene, which is responsible for the red colour of the tomato and of a few flowers, has not been shown to occur in animals. The xanthophylls are found both in green plant and in animal tissues.

All the carotenes function as provitamin A, the β-form being twice as active as the others. Lycopene and the xanthophylls* are inactive. The conversion to vitamin A, which apparently occurs in the liver, is a hydrolytic process.



The symmetrical molecule of β-carotene produces two molecules of vitamin A. In both α- and γ-carotenes one of the terminal ring-systems is slightly modified, so that only one molecule of vitamin is produced on hydrolysis. In lycopene and the xanthophylls, both ring-systems are modified, and no vitamin can be formed.

Animals derive the carotinoids laid down in their fatty tissues and milk- or egg-fats from the food, which usually contains both carotenes and xanthophylls. Some animals, e.g., the horse and cow, selectively store the hydrocarbons, others, e.g., the domestic fowl, the alcohols. Man apparently exercises no sharp selectivity, laying down carotinoids of different types as they appear in the diet. Still other mammals, e.g., the pig, sheep, goat and rat, deposit practically no carotinoid pigment in their adipose tissues or in their milk-fats, in spite of the presence of large quantities in the food. The wild rabbit lays down a colourless fat, but occasionally individuals are found with fat deeply coloured by xanthophyll. This is a characteristic inheritable according to Mendelian principles, yellow fat being recessive to white.

The concentration of the carotinoids present in fats is usually of the order of only a few parts per million, but recent developments

* With the exception of the recently discovered kryptoxanthine, $C_{40}H_{55}OH$, in which only one ring is hydroxylated.

in the methods of chromatographic analysis (fractional adsorption on active surfaces) have rendered practicable the separation and identification even of these extremely small amounts.

Antioxidants

Of particular importance in the preservation of fats are the traces of inhibitors or antioxidants which accompany them in the tissues, and which in some cases can be concentrated in the unsaponifiable fraction. These substances are considered in a later section (page 162).

Factors other than Species which influence the Composition of Fats

Although, as already seen, the composition of an oil or fat is governed primarily by the biological nature of the producing organism, various other causes, superimposed upon that of biological species, may operate to modify the structure of the fat laid down.

Temperature

It has frequently been contended that the chemical constitution and physical properties of the natural fats depend on the temperature at which they are formed, the tendency being for the production of solid, or at least relatively saturated fats, at high temperatures, and liquid oils containing a large proportion of highly unsaturated acids at low temperatures. Certainly it is a fact that in the vegetable kingdom the fats which are solid at 20° C. are practically all products of tropical or sub-tropical plants, while in the animal kingdom the great majority of the solid and semi-solid fats are products of the warm-blooded mammals and birds.* The converse, however, is by no means universally true, since highly unsaturated liquid oils are confined neither to cold climates nor to cold-blooded animals. Forty per cent. of the vegetable drying oils are of tropical or sub-tropical origin, and the warm-blooded marine mammals produce fats just as fluid and highly unsaturated as those of the cold-blooded fish.

TABLE 6.—*The influence of climate on the composition of linseed-oil.* (Ivanov[41])

Place.	Altitude (metres).	Climate.	Iodine-value of oil.
Davos (Switzerland) ..	1550	Cold and wet	189·6
Liebefeld (Switzerland) ..	550	„ „	188·4
Nolinsk (Russia)	—	„ —	185·1
Berlin tropical house ..	—	25–30°C. Saturated atmosphere.	92·6

* Storage of a fat which is not wholly or very largely fluid at the temperature of the organism would tend to hinder rapid mobilisation.

On the other hand, a number of cases exist in which temperature apparently influences degree of unsaturation within a species. Individual vegetable fats tend on the whole towards a greater uniformity of composition than do animal fats. Nevertheless, appreciable variation has been observed in the case of plants which grow over a comparatively wide range of temperatures. The data given in Table 6 represent an extreme case in which comparison was made between the iodine-values of linseed-oil obtained from seed of the same (Nolinsk) stock grown under abnormally warm or cold conditions. The same phenomenon has been observed with the moulds *Aspergillus niger* and *Rhizopus nigricans*[50], and with the timothy bacillus[54], all of which produce fats of decreasing iodine-value as the temperature at which they are grown increases.* It is also the case that within a particular species of salmon the iodine-value of the body-oil increases as one goes farther north[2], a finding which has been confirmed for other species of fish.

TABLE 7.—*Gradients of saturation and temperature in the fat of the pig.*
(Henriques and Hansen[30])

Location of fat.	Depth (cm.)	Body-temperature (°C.)	Barley diet		Maize diet	
			Iodine value.	Solidifying point. (°C.)	Iodine-value.	Solidifying point. (°C.)
Outer back fat 1	1	33·7	60·0	—	72·3	22·8
,, ,, 2	2	34·8	57·1	26·4	70·5	24·1
Inner back fat 1	3	37·0	51·8	28·0	65·5	25·7
,, ,, 2	4	39·0	50·6	27·7	64·2	25·6
Perinephric (kidney) ..	Body-cavity	39·9*	47·7	29·6	56·6	28·4
Caul	,,	39·9*	46·5	29·4	56·1	29·1

* Rectal temperature.

In certain of the warm-blooded animals the degree of unsaturation of the stored fat varies to a considerable extent with location in the body. Thus, the caul- and flare-fats of the ox are hard and high melting, that from tissues immediately beneath the skin is considerably softer, and that from the feet is a fluid known in commerce as " neatsfoot oil." In case of the pig, which deposits a thick layer of subcutaneous fat, a progressive increase in unsaturation and decrease in melting point can be observed as the skin is approached, a fact which has been attributed to a falling

* In the case of certain moulds, however, increase in temperature beyond the optimum for deposition of fat actually leads to an *increase* in the iodine-value of the fat produced,[51] which is in agreement with rate of deposition, rather then temperature, being the controlling factor.

body-temperature (Table 7). The existence of a gradient of saturation in the external fat has been confirmed by Dean and Hilditch[16] and by Callow[8] for the pig, and by Lea[44] for the ox.

On the other hand, no similar gradient exists in the hen, where superficial and internal fats from eight different depots have been found to possess approximately the same iodine-value[13], and no difference exists between the inner and outer layers of the *panniculus adiposus abdominalis* in obese human subjects[14]. Admittedly these are both cases in which the presence of feathers or of clothing reduces the gradient of temperature in the body, and in which the internal fat is already much softer than that of the sheep, ox or pig. It may be that softening of the external fat is only resorted to when the fat laid down in the interior of the body is of a fairly saturated type.

In Henriques and Hansen's experiments on the pig, use of a high external temperature or wrapping the animal in a sheepskin coat failed to eliminate the gradient of saturation, though decreasing the iodine-values of the outermost layers of the fat. Ellis and his collaborators[17, 27] showed that in the growing pig a low rate of deposition produces a soft fat, and rapid deposition a hard fat (page 23). Callow[8], extending this principle, found a parallelism between the rate of deposition of fat in various depots and the rate of decrease in iodine-value with age, and showed that this explanation accounts for certain differences in iodine-value which cannot be explained by variation in body-temperature. It appears therefore that temperature of deposition is not the sole, and may not be the most important, factor governing the composition of depot fats in warm-blooded animals. The whole question requires further study.

Age and Diet

The most important single factor which modifies the composition of the fat laid down by an animal is undoubtedly the nature, and particularly the fat-content, of the diet. Animals as well as plants are capable of synthesising fat from carbohydrate or protein, so that an animal receiving a diet practically free from fat will develop a certain amount of adipose tissue, which will be of a composition characteristic of the species. When, however, large quantities of fatty material are fed, the body- and milk-fats may be radically altered. The process by which the fat of the food is utilised presumably involves hydrolysis of the ingested fat, followed by combustion of some of the resultant acids and building up again of the residue, together with synthetic material, into the glycerides and phosphatides which are deposited in the tissues. Some selection or control is undoubtedly exercised by the organism, since not all acids present in the food necessarily appear in the fat. As an example of the extent to which modification of the body-fat can occur, data obtained for the rat may be quoted (Table 8). In this case up to 60 per cent. of the total calories of the diet were fed as fat.

TABLE 8.—*Effect of diet on the body-fat of the rat.**

Food-fat.	Fat-content of diet. Per cent.	Iodine-value of food-fat.	Iodine-value of body-fat.
Soya-bean oil	37·2	132·3	122·5
Maize-oil	37·2	124·3	114·2
Cottonseed oil	37·2	108·1	107·4
Arachis-oil	37·2	102·4	98·4
Hydrogenated cottonseed oil ..	37·2	78·8	81·8
Lard	37·2	63·2	71·7
Butter-fat	37·2	35·8	55·5
Coconut-oil	37·2	7·7	35·3
Basal ration	—	—	59·1
Cottonseed oil	3	108·5	62·7
,, ,, ...	6	108·5	75·9
,, ,, ...	9	108·5	80·5
,, ,, ...	12	108·5	94·2
Hydrogenated cottonseed oil ..	3	71·0	63·3
,, ,, ,, ..	6	71·0	68·5
,, ,, ,, ..	9	71·0	75·0

* From data by Anderson and Mendel[2] and by Ellis, Rothwell and Pool.[11]

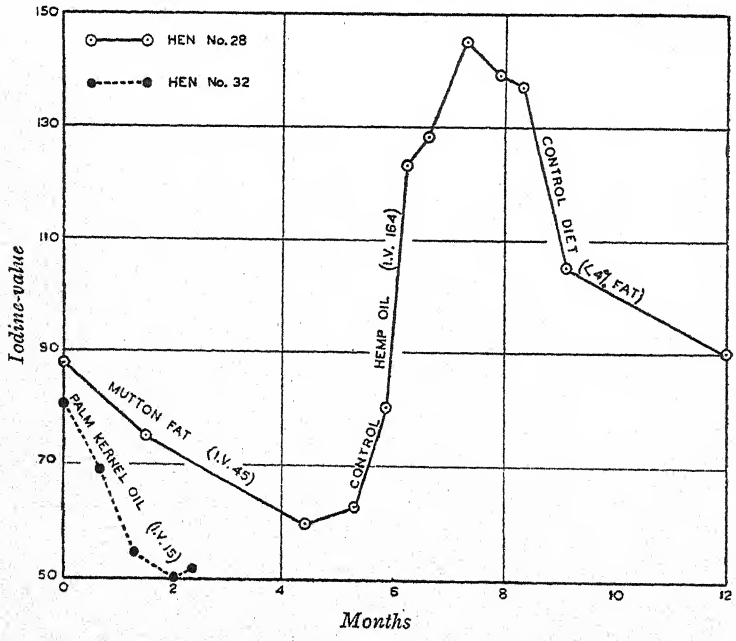


FIG. 1.—*Effect of diet on the body-fat of the hen. (From data given by Cruickshank.*[13])

The body and egg-yolk fats of the hen also respond rapidly to the incorporation of large amounts of fat in the diet. Periodical removal of samples of abdominal fat without injury to the bird has shown that the "normal" iodine-value of 80–90 obtained on a control diet containing less than 4 per cent. of fat can be either increased or decreased by the ingestion of appropriate fats in the ration (Fig. 1). On the other hand, the yolk-fat could not be saturated to an iodine-value below 80, though figures approaching 130 were recorded on the hemp-oil ration (Table 9). The mean molecular weight of the yolk-acids on the palm-kernel diet showed no signs of deposition of the acids of lower molecular weight characteristic of this fat.

Quality in the Fat of Pork

Quality in the fatty tissues of the carcase from a healthy and properly finished pig is mainly a question of consistency and susceptibility to oxidative rancidity.

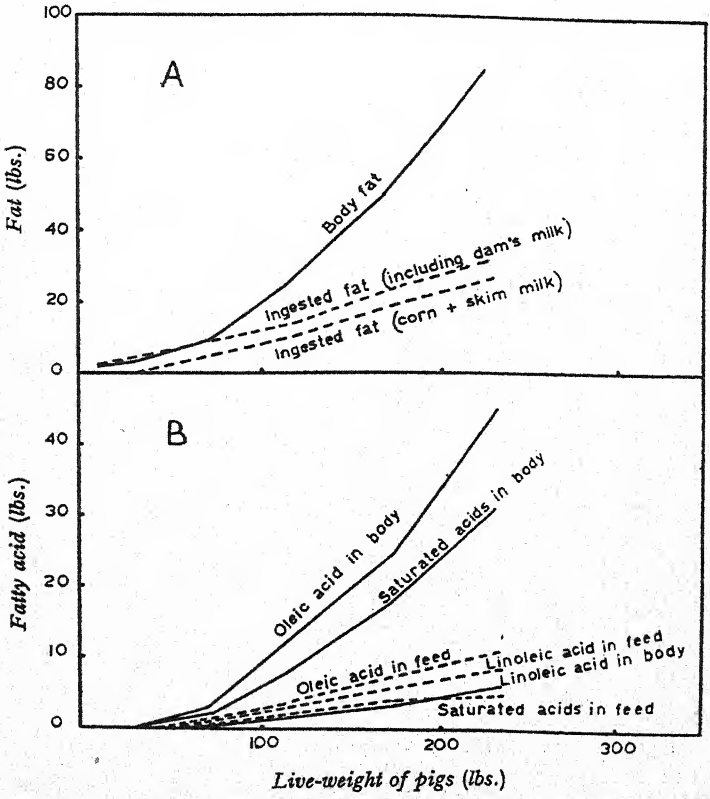


FIG. 2.—*The velationship between (A) food-fat and body-fat, and (B) individual acids in the food- and body-fats.* (Ellis and Hankins.[17])

TABLE 9.—*Effect of diet on the constituent fatty acids of egg-yolk fat* (Cruickshank[13]).

	Control mash.	Fishmeal-free mash.	Control+ palm-kernel (I.V.15)	Control+ palm-oil. (I.V.53·1)	Control+ mutton-fat (I.V.44·5)	Control+ linseed-oil (I.V.180·6)	Control+ hemp-oil (I.V.163·7)	Hempseed alone* (I.V.163·7)
Iodine-value	84·4	80·0	80·5	85·9	84·0	123·1	115·7	127·2
Mean molecular weight	280·5	283·0	280·3	281·4	284·0	281·8	282·1	280·6
Saturated acids.. ..	31·4	31·2	30·3	27·8	29·5	23·9	24·3	21·4
Oleic acid	46·7	51·4	51·9	49·8	50·8	33·8	28·8	26·7
Linoleic acid	19·0	15·0	16·1	21·7	16·9	24·9	41·7	41·9
Linolenic acid	2·9	2·4	1·7	0·7	2·8	17·4	5·2	10·0

* A diet higher in protein and very much lower in carbohydrate than the control.

Consistency

The usual fault is abnormal softness of the fat, which results in carcases and joints which are soft and flabby and of greasy, unattractive appearance. In such cases loss of fat from the tissues during cooking is excessive, and the rendered lard lacks body and may even be fluid at ordinary temperatures. Bacon from pork with soft fat is sometimes difficult to slice.

The pig derives its body-fat largely from the carbohydrate and fat of its food, protein being rarely fed in sufficient quantity to be of much importance as a source of fat. In the young animal of 30 lb. weight only about 0·03 lb. of fat is deposited for each 1 lb. increase in total weight, but by the time 230 lb. is reached the rate at which fat is deposited has increased to 0·65 lb. per lb. gain[17]. A diet containing about 4 per cent. of fat therefore supplies more of this material than the young pig deposits, but provides only a small fraction of the fat which the larger animal lays down (Fig. 2A). The fatty acids of the food-fat thus enter to a steadily decreasing extent into the composition of the body-fat of the growing pig, and since the fat of the food (Table 10) is usually liquid and more highly unsaturated than that synthesised from carbohydrate by the pig (iodine-value 50–55), it follows that the body-fat becomes progressively harder with increasing age and weight.* As the pig becomes still older and the rate at which fat is deposited slows down, it is probable that a reversal of the hardening occurs, since the fat of sows two or three years old has been found to possess higher contents of linoleic and of highly unsaturated acids than that of the seven months (200 lb.) animal on a similar diet[16]. The fat of the ox on a ration of corn, linseed-meal and hay has been found to increase in iodine-value and decrease in melting point with increasing age and fatness[47].

The characteristics of the body-fat of the pig are therefore determined by the amount and degree of unsaturation of the fat in the diet, and by the rate at which fat is deposited. Coconut-oil in large quantity will reduce the iodine-value of the back fat to 30[23] while liquid fats of iodine-value greater than 100 can be obtained by feeding linseed and similar oils. In these latter cases the most marked change in composition is a progressive increase in linoleic acid as the fat becomes softer (Table 11). This is of interest in view of the fact that the amount of linoleic acid laid down appears never to exceed that given in the food (Fig. 2B), and though some doubt may exist as to whether or nor certain animals are *totally* unable to synthesise linoleic acid[7], it certainly seems that this acid is not synthesised in quantity by the pig for deposition in the stored fat as are oleic and the saturated acids.

* On diets of very low fat-content, e.g., 0·5 per cent, the fat even of young pigs is hard[20], and on diets of very high fat-content, hardening with age becomes negligible.

TABLE 10.—*The fat-content and iodine-value of the extracted fat of various feeding stuffs.**

Food.	Fat-content per cent.	Iodine-value	Food.	Fat-content per cent.	Iodine-value
Barley-meal	1·6	124·6	Peanut meal	9·0	93·7
„ „	1·6	116·7	Shelled peanuts	47·6	93·7
Oats (crushed)	3·3	100·5	Cottonseed meal	6·8	107·4
Wheat-middlings	4·3	128·4	Separated milk	0·2	33·0
„ „	3·9	126·7	Sago pith meal	0·2	75·3
„ „	4·2	115·4	Tankage	10·9	64·5
Bran	3·3	127·6	Fish meal	11·0	124·8
Maize	4·3	126·0	„ „	2·2	151·1
Rice polish	9·7	100·0	„ „	2·2	169·9
Rice-bran	14·8	100·0	Linseed oil	—	170–185
Brewers' rice	0·8	100·0	Coconut oil	—	8–10
Soya-bean meal	17·5	128·0	Whale oil	—	110–150
„ „ „ (extracted) ..	0·5	134·8	Cod liver oil	—	155–170
Acorns	4·4	100·7	Menhaden oil	—	140–190
Peanuts (whole)	33·1	93·7			

* From data by Callow[10] and by Hankins and Ellis.[37]

Fat laid down on a softening diet can be hardened to some extent by changing to a diet low in fat, or to one containing more saturated fat, but the process—depending as it does in the fattening animal largely on dilution with the harder fat—is slow, and it is extremely difficult to eliminate highly unsaturated acids which have been deposited on the original diet.

TABLE 11.—*The effect of ingested fat on the composition of pig's fat.* (Ellis and Isbell[18])

	Diet No. 1.	Diet No. 2.	Diet No. 3.	Diet No. 4.	Diet No. 5.	Diet No. 6.
Days on diet ..	84	188	80	277	52	53
Gain in weight (lb.)	178	201	58	149	65	98
Carcase grade ..	Hard	Hard	Oily	Oily	Oily	Oily
Fat—						
Melting point (°C.)	39·7	37·5	22·5	< 5	22·0	28·1
Iodine-value ..	52·6	58·8	84·1	91·8	90·7	100·6
Fatty acids—						
Oleic	58·4	54·4	56·8	64·6	40·4	38·8
Linoleic	1·3	7·0	19·5	19·7	31·8	38·2
Linolenic ..	—	—	—	—	0·2	0·5
Arachidonic ..	0·02	0·06	0·12	0·05	0·08	0·05
Myristic	1·8	0·6	0·4	0·1	0·7	0·3
Palmitic	26·4	25·2	15·5	10·4	17·3	14·1
Stearic	12·1	12·7	7·4	4·9	9·4	8·0
Arachidic ..	—	—	0·2	0·3	—	—

DIET No. 1.—Brewers' rice and tankage. Very low in fat.
DIET No. 2.—Maize and skimmed milk. Fairly low in fat.
DIETS Nos. 3 AND 4.—Peanuts alone. High in fat.
DIETS Nos. 5 AND 6.—Soya-beans alone. High in fat.

TABLE 12.—*Average characteristics of various grades of pig's fat.* (Hankins and Ellis[27])

Grade.	Refractive index.	Iodine-value.	Melting point.
Hard	1·4593	63·0	38·0
Medium hard ...	1·4599	68·0	36·5
Medium soft ..	1·4603	71·0	35·0
Soft	1·4611	77·5	31·0
Oily	1·4623	88·0	24·0

Softness in pork-fat has been measured by various chemical and physical methods. Ellis and his collaborators, who have done much work in this field, made use of the refractive index, iodine-value and melting point of the back fat. These values were calibrated against the reports of a grading committee which examined

the carcases chilled at 34°-35° F. for 72 hours after dressing. Average data for the various grades are given in Table 12. Iodine-value and refractive index were found to provide satisfactory measures of the firmness of the adipose tissue, but titer and melting point did not always move in harmony with changes in the other values or in consistency. Certainly the iodine-value in particular has proved a useful general guide to firmness, and has been widely used for this purpose. The assumption that the softness of a fat varies directly with the total concentration of double bonds present is, however, at best an approximation, and direct measurement of hardness by a physical method appears preferable. Such methods have been proposed for butter-fat[22] and for partially hydrogenated fats[25], and in the latter case at least it has been demonstrated that iodine-value and melting point can both give completely erroneous estimates of hardness.

Susceptibility to Oxidation

Practically no quantitative data have been obtained concerning the effect of diet on the susceptibility of the body-fat to oxidation. Soft or oily carcases frequently appear to be abnormally liable to rancidity, as indeed might be expected from the increasing susceptibility to oxidation displayed by individual fatty acids as the degree of unsaturation increases (page 86). Thus, the fat of pigs receiving considerable amounts of whale-oil or low-grade fish-meal has been found to be soft and to become rancid very quickly[4].

TABLE 13.—*The influence of cod-liver oil in the diet on the susceptibility to oxidation of pig's fat.*
(Lea[45])

Diet.	Iodine-value.	Relative resistance to oxidation.	
		Experiment 1.	Experiment 2.
No. 1. Without cod-liver oil ..	67·0	1·00	1·00
No. 2. Cod-liver oil to 100 lb. weight	66·1	0·30	0·30
No. 3. Cod-liver oil to slaughter (200 lb.)	69·2	0·21	0·20
No. 4. Cod-liver oil to slaughter (200 lb.)	71·3	0·21	0·19

It has become a not uncommon practice during the winter, when fresh green food and sunlight are available only in greatly restricted amount, to supplement the diet of domestic animals, particularly pigs and poultry, with cod-liver oil. Preliminary experiments by Lea[43, 45] showed that inclusion of this oil in the diet, even in quantity too small to affect appreciably the iodine-value of the body

fat, markedly increased susceptibility to oxidation. Withdrawal of the oil when the pigs had reached half weight (100 lb.) improved the fat, but failed to restore its level of resistance to that possessed by the fat of control animals which had not received the oil (Table 13 and Fig. 3). This result is presumably due to deposition by the pig in its body-fat of highly unsaturated, highly unstable fish-oil acids in quantities too small to be apparent above the normal variation

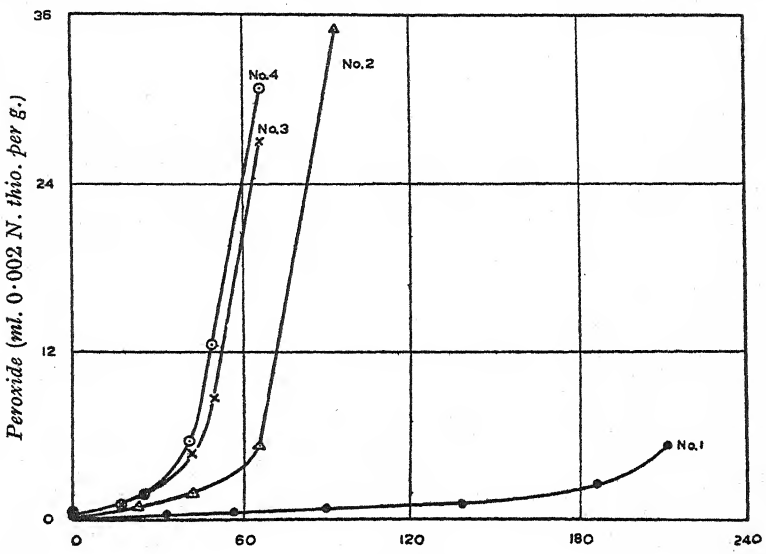


FIG. 3.—*Relative susceptibility to oxidation of the back fat of groups of pigs receiving* (1) *basal ration*, (2) *basal ration, plus* 1 *oz. cod-liver oil to* 100 *lb. weight*, (3) *and* (4) *basal ration plus* 1 *oz. cod-liver oil to killing weight* (200 *lb.*). (Lea[45]).

ie iodine-value from animal to animal, but sufficient by their rapid oxidation to destroy the natural inhibitor and greatly reduce the induction-period of the fat. Pigs' fat normally contains traces (up to 0·4 per cent.)[6] of highly unsaturated acids, and larger amounts (1-2 per cent.) have been found in the fat of old sows[16]. These quantities are undoubtedly increased when fish-oils are fed in the diet.

It seems therefore that some method of increasing the content of fat-soluble vitamins in the diet, without at the same time adding highly unsaturated fatty acids, is desirable. Halibut-liver oil, being much richer in vitamins than cod-liver oil, could be fed in much smaller quantity. Alternatively, a fat-free concentrate, such as the unsaponifiable fraction of cod-liver oil, or irradiated yeast or calciferol (as source of vitamin D), could be used. Hydrogenation of cod-liver oil might perhaps provide the most practical solution. This process destroys the highly unsaturated acids and, if carried out

under high pressure and at low temperature [55, 56], the vitamin D content of the oil is practically unaffected and the vitamin A not greatly reduced.*

Up to the present only the fatty-acid composition of the fat has been considered. It is, however, quite reasonable to suppose that nutritional factors may also affect the antioxidant-content of the fatty tissues, since a number of cases are known in which unsaponifiable constituents associated with the fat of the food enter the body- or milk-fat. The possibility thus arises of considerable variations in susceptibility to oxidation, apart from those due to fatty-acid composition. Direct determination of susceptibility should therefore be made, either on the extracted fat or on the fatty tissue, in addition to chemical examination of the fat.

Variation in the Fat of Butter

Butter-fat varies in composition with the age, breed and diet of the cow. The most important properties affected are colour, vitamin-content and consistency.

Colour and Vitamin-content

The yellow pigment of butter-fat consists of β-carotene, together with a minor proportion of xanthophyll, both derived directly from the diet. Vitamin A produced by the cow from the carotene of the food, and a small amount of vitamin D, are also present. The content of vitamin D is highest in the summer, when both cow and

TABLE 14.—*The influence of breed and diet on the colour of butter-fat.*†

Ration.	Ayrshire.		Holstein.		Jersey.	
	Yellow	Red.	Yellow	Red	Yellow	Red
Carotene-poor (cottonseed hulls and meal, timothy hay, silage, white and yellow corn, beet, etc.)	1·8	0·4	5·7	0·9	7·0	1·5
Carotene-rich (mixed grain, with carrots, green lucerne hay, fresh pasture grass, etc.) ..	20·0	1·2	38·0	1·5	55·0	1·8

† From data given by Palmer[49]. The figures, which are yellow and red Lovibond units per 1-in. cell are average values, fats from different animals giving rather variable results.

pasture are receiving sunlight. The activity of winter milk and butter can be increased by feeding the cow with material naturally rich in vitamin D, such as cod-liver oil or its unsaponifiable fraction; by exposing the cow to sunshine or other source of ultra-violet

* Hydrogenation of cod-liver oil also destroys its toxicity for herbivorous animals. Irradiated hydrogenated vegetable oil is another possible source of vitamin D.

light; by feeding irradiated materials, e.g., ergosterol or yeast; by irradiation of the milk; or by direct addition of vitamin D concentrates.

The colour and vitamin A content of butter vary with the amount of carotene in the diet, and therefore tend also to be greatest when the cow is receiving fresh green food (Table 14). Average vitamin A and D activities as determined by biological assay have been given for summer butters as 27·2 (June, 35·9) and 0·49 (June, 0·80), and for winter butters as 15·4 (April, 11·3) and 0·16 (January, 0·07) international units per gram respectively.* Artificially dried grass is much more effective than ordinary sun-cured hay or silage in maintaining the colour and vitamin A content of winter butter at a high level[24]. Preformed vitamin A in cod-liver oil, etc., increases the vitamin-content without appreciably increasing the colour of the butter.

TABLE 15.—*Seasonal variation in the carotene and vitamin A content of butter.**

Breed.	Carotinoid (γ per g.)	Baumann et al. [5]		Gillam et al. [24]	
		Summer pasture.	Winter diet	Summer pasture.	Winter diet
Holstein ..	Carotene	6·6	5·2	—	—
	Vitamin A	15·1	10·2	—	—
Guernsey ..	Carotene	17·0	10·3	11·4	7·3
	Vitamin A	8·5	6·8	9·5	5·4
Ayrshire ..	Carotene	5·5	4·8	4·1	2·7
	Vitamin A	12·2	8·4	11·8	6·6
Jersey ..	Carotene	10·7	7·1	—	—
	Vitamin A	11·5	7·1	—	—
Shorthorn	Carotene	—	—	2·9	2·5
	Vitamin A	—	—	8·4	5·8
Friesian ..	Carotene	—	—	4·7	3·5
	Vitamin A	—	—	12·1	6·1

* Average values; variations between cows of the same breed on the same diet are of the order of 100 per cent.

Cows of different breeds vary greatly in the colour of the butter they produce on the same diet, the Channel Island types giving more deeply pigmented butters than the others (Tables 14 and 15). Vitamin A, which is practically colourless, does not vary in the same way, so that the most deeply coloured butters do not necessarily possess the greatest total biological activity. Butter from a shorthorn, for example, on a high-carotene diet might have the same colour as butter from a Guernsey on a low-carotene diet, but the former would be much richer in vitamin A. Assuming 0·6γ of vitamin A as biologically equivalent to 1γ of carotene (the efficiency

* MORGAN, R. S. and PRITCHARD, H. *Analyst*, 1937, **62**, 354.

of the conversion in the animal is not accurately known), there appears to be no very great difference in total activity of the butters from different breeds of cow on the same diet[5]. The efficiency of translation of the carotene of the diet to the carotene and vitamin A of the milk is only of the order of 1–3 per cent[5].

Consistency

Consistency, plasticity, texture and "spreadability" of butter are not functions of the hardness of the fat alone, but are influenced in a marked degree by other factors, such as the cooling of the cream, and the conditions of churning and of working the butter. Nevertheless, the composition of the fat has an important influence on the physical properties of the butter. The factor which largely determines hardness is undoubtedly the diet of the cow, but the age of the animal appears also to play some part, a progressive increase in unsaturation and softness of the fat being detectable over a period of years[15].

The most important dietary change is usually from winter feed to pasture in the spring, and back to winter feed in the autumn. Fresh pasture produces a fairly soft fat of iodine-value in the neighbourhood of 40, or even higher.* Winter diets of roots, hay, silage, coconut or palm-kernel cake, and oil-cake meals of low fat-content produce hard butters. Thus, average iodine-values of Danish butters have been found to increase from 29·9 in December to 41·4 in August, and of Swedish butters from 31·0 in January and February to 40·0 in August and September. Butters from English shorthorn cows showed an abrupt increase in oleic and linoleic acids in the fat when the cows returned to pasture[15]. The major part of the change occurred within a week or two of the change in diet, though usually a slight drift was also noticeable throughout the season. Iodine-values ranged from 36·5 in February to 44·2 in June[36].

The magnitude of this seasonal variation in consistency naturally depends on the composition of the winter diet, inclusion of large amounts of highly unsaturated oils, such as linseed, sunflower, sesame or rape, leading to the production of fats softer than normal summer butters (Table 16). Hansson and Oloffson[28] have come to the conclusion that, for the production of a winter butter of suitable consistency, the feeding cake should contain about 5–6 per cent. of oil.

Susceptibility to Oxidation

It might reasonably be expected that changes in fatty-acid composition and possible variations in antioxidant-content might result in some degree of correlation between the susceptibility to oxidation of the fat and the diet of the animal. With the exception of one or two isolated observations (page 204), no attempt appears to have been made to establish such a connection, but the observation that butters from stall-fed cows which had received cod-liver oil showed considerable loss of vitamin A after cold storage for 20 months

* The small amount of fat in grass is highly unsaturated.

TABLE 16.—*The influence of diet on the composition of butter-fat.**

	Basal winter ration	Basal + 8 oz. linseed-oil	Basal + 8 oz. rape-oil	Basal + 8 oz. cod-liver oil	Summer pasture	Summer pasture
Iodine-value of fat	34·5	46·0	44·5	54·1	41·6	45·0
Butyric	4·4	4·2	3·6	2·1	3·3	3·1
Caproic	2·2	2·0	1·6	0·9	1·3	1·7
Caprylic	2·4	1·3	1·3	0·5	1·2	0·7
Capric	3·8	2·3	1·6	1·2	2·2	1·8
Lauric	4·4	3·1	2·8	3·1	4·0	3·2
Myristic	10·9	8·4	8·6	6·4	10·4	7·1
Palmitic	23·1	21·8	18·1	22·7	26·1	22·8
Stearic	12·6	9·9	13·8	6·7	6·5	12·5
(as) Arachidic	0·7	0·6	0·5	0·6	—	0·7
Oleic	28·9	39·3	39·7	43·3	40·9	41·3
(as) Linoleic	5·6	5·9	3·7	4·8	4·1	5·1
(as) $C_{20\text{-}22}$ unsaturated ..	1·0	1·2	1·0	7·7	trace	trace
(as) Erucic	—	—	3·7	—	—	—

* From data by Hilditch and Thompson[40], Hilditch and Sleightholme[34] and Dean and Hilditch.[23] Recent work[28] has established the fact that normal butter-fat contains traces of decenoic acid, about 1 per cent. of myristoleic and 4–5 per cent. of palmitoleic acid. Correcting for these, the values for oleic and palmitic acids given in the table are approximately 5 and 2 per cent. too high. Minute traces of behenic, lignoceric and "cerotic" (C_{26}) acids (included in the analysis as arachidic acid) are also present,[29] the latter presumably derived from the wax esters of the grass eaten by the cattle.

while others from pasture-fed animals showed little loss in three years[12], is significant, particularly in view of the undoubted entry of highly unsaturated fish-oil acids into the fat (Table 16).

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PART II.—RANCIDITY: OCCURRENCE AND TYPES

Recognition by Taste and Smell

Investigation of the problem of rancidity from a scientific point of view is rendered more difficult by the fact that the ultimate standards to which all data must be referred are based on taste and smell, the most elusive and least clearly defined of the five senses.

The physiology of taste and smell is reasonably clear. Taste is due to stimulation of specific nerve-endings in the tongue, where cells specially sensitive to sweetness, sourness, saltness and bitterness tend to be localised in particular areas. Sensitivity is not very great. For sips of 1 ml. the threshold values for taste are of the order of 7 mg. for sugar, 1·5 mg. for salt, 0·2 mg. for tartaric acid, 0·04 mg. for caffeine, 0·016 mg. for quinine and 0·012 mg. for saccharine, at which point the limit of sensitivity has almost been reached[1]. Response to taste stimuli, however, varies greatly in different individuals. Tests on 59 persons tasting 5 ml. of solution showed that the threshold for salt (sodium chloride) ranged from 0·0008 to 0·2048 M, for sweet (cane-sugar) from 0·0004 to 0·1024 M, for sour (lactic acid) from 0·0002 to 0·0128 M, and for bitter (caffeine) from 0·0002 to 0·0128 M. Of the whole group only five persons showed a low, five a medium and two a high threshold of taste for all four substances, the remaining 47 giving mixed reactions[2].

The sensation of smell depends on stimulation of the olfactory nerve-endings which are concentrated in a small area situated high up in the nose behind and below the eyes, and from which the path to the brain is short and direct. The sense of smell is capable of much finer perception and discrimination than that of taste, and in fact the majority of the stimuli which are received from food and attributed to "taste" are actually due to smell. The sensitivity of smell (and hence of "taste") is very great. About 100 ml. of air are required for detection of an odour of ordinary strength, less if the odour is strong. Approximate threshold concentrations in air (mg. per litre) for some of the more odorous chemicals have been given as follows:—chlorophenol, 4×10^{-6}; ionone, $5 — 10 \times 10^{-8}$; artificial musk, $5 — 40 \times 10^{-9}$; and vanillin, $2 — 5{,}000 \times 10^{-10}$. Amounts of the order of 10^{-12} gram can thus be detected in some cases. "Pungency," which is probably a sensation of pain, appears to be the same for such dissimilar substances as ammonia, formaldehyde and acetic acid, but at high dilution the true odour can usually be perceived.

It is possible for the senses of taste and smell to be developed to a remarkable standard of sensitivity and consistency, as, for example, in professional wine- and tea-tasters. Nevertheless, the ordinary method of assessing flavours and preferences in food, and

of identifying " off " flavours and taints, by permitting numbers of people to taste numbers of samples, leaves much to be desired. This is due to the fact that the stimuli provided by the gustatory and olfactory nerves are invariably combined in the brain with other sensory qualities, such as those of warmth, coolness, roughness, astringency and oiliness, as conveyed from the tactile organs of the mouth and nose. At the same time they tend to be confused by moods, feelings and associations of which the observer is largely unconscious, and over which it is very difficult for him to exercise control. The connection between the senses of sight and " taste " is easy to demonstrate. Experiments in which untrained observers are asked to assign names to fruit-flavoured jellies which they are not permitted to see, or which are coloured in a manner inappropriate to their flavours, give a low percentage of correct results, and opinions on the flavours of jellies of identical composition but different colours are equally unreliable. Other disturbing factors encountered in applying tasting tests are those of contrast and fatigue; distilled water will taste sweet if it follows something sour or bitter, and sensitivity to slight differences in flavour rapidly falls off as a series of samples is tasted. The general environment, too, has some effect on the reaction towards a flavour, and probably no person can make an unbiased comparison of a new flavour against a well known one.

Sensitivity and consistency in reporting differences and preferences in odour and flavour vary greatly from one person to another. It is therefore of advantage to select for a tasting panel individuals who are above the average in these respects. Tests for making such a selection can easily be devised[2, 4]. The efficiency of a panel in classifying samples falls off rapidly as fatigue reduces sensitivity, and in the case of fats one rancid sample may disable the panel for an appreciable time. Orange- or lemon-juice is said to be a useful restorative under such circumstances! Samples for tasting, with the exception of the control, should always be indicated by numbers only, otherwise suggestion may lead to unreliable results. The inclusion of additional fresh control samples in the series serves as a useful check.

Probably the greatest difficulty encountered in classifying samples by taste and odour is the impossibility of assigning numerical values to the results, or in conforming to any permanent standard of reference. In working on rancidity it is therefore preferable to make use of chemical methods wherever possible, in order to obtain the necessary degree of accuracy and reproducibility. The methods used can then be calibrated against odour and flavour for each set of experimental conditions.

Types of Spoilage

Fat is present in the majority of foods, and in many cases deterioration of this constituent during the interval between

preparation and consumption may result in spoilage. The losses which are continually being incurred from this cause cannot readily be estimated, but must certainly be very large.

The importance of rancidity is not limited to foods of high fat-content, since it can appear under conditions which largely inhibit other forms of deterioration. Thus, foods capable of resisting bacterial spoilage for long periods may ultimately become unpalatable through chemical changes in the fat. Lea[3] has found that biscuits prepared from wheat flour without addition of shortening develop an unpleasant flavour after prolonged storage, owing to the development of oxidative rancidity in the small amount (< 1 per cent.) of fat present. On the other hand, the proteins of meat and fish, for example, are decomposed so rapidly by micro-organisms at ordinary temperatures that rancidity becomes of only secondary importance in rendering the material inedible, even when large amounts of readily oxidisable fat are present. Methods of preserving food, other than canning or vacuum-packing, tend, however, to control bacterial spoilage much more effectively than chemical changes in the fat, with the result that defects in flavour and appearance originating in the fat assume relatively greater importance in the frozen, dried or salted product than in the fresh material. A list of common foods in which deterioration of the fat is of importance would include lard and other animal and vegetable shortening and frying fats, salad-oils and dressings, medicinal preparations of cod- and halibut-liver oils, biscuits and pastries, cereals and flour, milk, cream, butter, dried milk and infant-foods, ice-cream, chocolate and caramels, potato-chips, bacon and chilled meat, and frozen and salted fish.

The term "rancidity" has been employed in the past, either in a general sense to indicate deterioration in odour or flavour without any attempt to specify the cause, or, alternatively, as applying exclusively to some particular type of spoilage. In the latter case "rancidity" has usually been reserved for hydrolytic changes produced in butter and similar fats by the action of enzymes or micro-organisms, while "tallowiness" has been used to denote oxidation.

This confusion in nomenclature has resulted in the appearance of a number of apparently contradictory statements in the literature. It has been claimed, for example, that micro-organisms are alone responsible for rancidity, or alternatively that rancidity can arise only as a result of the joint action of air and light, and that micro-organisms are without effect on its development. For the purpose of this report, "rancidity" is defined in its widest sense simply as a deterioration in odour and flavour which develops in fats or in the fatty constituents of food-stuffs on keeping. For convenience of treatment the possible causes of rancidity have been classified under several headings:—(*a*) absorption of odours; (*b*) action of enzymes

present in the tissues; (*c*) action of micro-organisms; and (*d*) atmospheric oxidation. These will be considered separately, but it should be remembered that a rancid condition may sometimes result from the joint operation of two or more of these causes.

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PART III.—THE LESSER CAUSES OF RANCIDITY

Absorption of Odours

One of the difficulties in the commercial handling and storage of foodstuffs is to prevent tainting by odours given off from packing materials, new paint-work, fuel-oil, etc., or from other goods stored in the vicinity. Foods of high fat-content and mild flavour are particularly susceptible to spoilage of this kind, probably owing to the ready solubility of the majority of natural odorous substances in a fatty substrate. As cases in point may be mentioned the tainting of butter, egg-yolk and the fat of meat by fruits.

Eggs

Egg-yolk, which contains over 30 per cent. of fat, readily absorbs foreign odours, and " cold-storage " tastes can easily be produced in this way. Strawboard fillers and separators used for packing have been known to cause the trouble. Eggs stored in proximity to growing colonies of actinomyces rapidly absorb the characteristic " musty " odour produced by these micro-organisms, and the flavour thus acquired is not lost on cooking[9]. Actinomyces are widely distributed in the soil, on grasses and straw, etc., and easily find access to cold stores, where some strains are still capable of growth at the temperatures used for the storage of eggs.

Heavy losses from tainting by odours from citrus fruits were experienced some years ago in shipments of eggs arriving in England from South Africa[18]. The taint, which could not usually be detected until the eggs were broken, varied from a barely perceptible flavour to one strong enough to render the eggs uneatable. The taste was not that of the fruit itself, but of the essential oils of the skin. In this instance the trouble was traced to storage prior to shipment in buildings which were also used for fruit, the odours of the latter being permitted to diffuse into the chambers containing the eggs. Further contamination occurred in some cases during the voyage from the woodwork, dunnage, and insulation of chambers which had previously been used for fruit. Care in the construction and filling of stores and holds, and the practice of deodorization after a chamber has been used for highly odorous commodities, have greatly reduced the incidence of gross spoilage of this type.

Butter

One of the forms of absorption of odour experienced in the storage and transport of butter, is that of " wood " or " timber " taint, in which a resinous flavour, confined to the edges and outermost layers of the slab of butter, is caused by absorption of volatile terpene substances from boxes made from unsuitable varieties of wood. This fault is of some importance to the Australian and New Zealand exporter, since a number of the cheaper local woods are

of this type. In practice it has been found possible markedly to reduce the risk of taint by spraying the boxes successively with solutions of casein-borax and formaldehyde, which has the effect of sealing the surface of the wood under a hard and difficultly permeable coating[22]. Some success has also resulted from lining the boxes with combinations of parchment and metallic foil, the latter serving to exclude the tainting vapour[20].

TABLE 17.—*The absorption of limonene from a saturated atmosphere by butter-fat.*

(Gane[7])

Temperature (°C.)	20	10	5	0	−10	−20
Vapour-pressure of limonene (mm. Hg.)	1·01	0·54	0·39	0·33	0·17	0·08
Limonene absorbed (mg. per 100 sq. cm. per min.)	3·60	1·76	0·96	0·59	0·18	0·08
Ratio of rate of absorption to vapour-pressure	3·57	3·26	2·46	1·78	1·08	0·96

Gane[7] has carried out some investigations on the absorption by butter-fat of *d*-limonene ($C_{10}H_{16}$), which is the chief constituent of the oil of orange-rind. The avidity with which this strongly odorous substance is taken up from the air by a free surface of fat is shown in Table 17. Diffusion into solid butter-fat at —10° C., however, was found to be very slow, the whole of the limonene absorbed (0·82 g. per 100 sq. cm.) during exposure for 14 days to a saturated atmosphere being confined to the outermost 1 mm. of the fat. Limonene has also been found to taint the yolk of eggs exposed to its vapour, though the surrounding white, which contains practically no fat, failed to retain the taste[21]. Here again only the outer few millimetres of the yolk were affected, diffusion in the fatty emulsion being relatively slow.

Meat

The growth of slime-producing bacteria on moist surfaces of meat stored at ordinary temperatures or in the chilled state, produces an unpleasant "tainted" odour which may unfavourably affect the flavour of the fat of sound meat stored in the same chamber. Lea[12] has carried out some experiments in which fresh beef-fat, embedded in paraffin wax in order to expose only one surface to the air, was stored at 0° C. in proximity to tainted meat. A deterioration in the flavour of the fresh fat, which persisted after cooking, was noticeable after exposure for a few hours. The taint was confined to a thin superficial layer, samples taken at a depth of more than a few millimetres from the surface being perfectly sweet. A covering of connective tissue was found to afford some protection to the fat. In other cases more prolonged storage resulted in deeper penetration of the taint. An odour and taste

due to hessian wraps, used to prevent chafing, has been observed on several occasions in the fat of commercial chilled beef.

Prevention of Tainting

Spoilage by absorption of odours can, of course, be eliminated completely by careful segregation of susceptible products from tainting materials. Such conditions are sometimes difficult to attain in practice, as, for example, in the overseas transport of food, where adjacent refrigerated spaces may be required for products of widely different types, or where a particular hold or chamber is used on successive occasions for different commodities. Alternative methods consist in protecting the product by means of a wrapper impervious to the tainting vapour, or by destroying the latter in the air. Many foods are already packed with the object of reducing loss of moisture, but some at least of the materials effective for this purpose (e.g., waxed paper) do not adequately exclude tainting vapours. Metallic foils, as used for the wrapping of cheese, are practically impermeable to all vapours, but are relatively expensive in the weights necessary for adequate mechanical strength. Parchment paper backed by metallic foil is less expensive and quite effective.

Destruction of objectionable vapours by means of ozone is of particular value in purifying the air of chambers after use for odorous commodities, before the introduction of others which are susceptible to taint. The gas is also used to a limited extent during storage, as, for example, in the commerical storage of eggs, where there is the joint object of destroying odours and inhibiting microbial growth (page 71). Care, however, is necessary under these conditions because of the susceptibility of foods, particularly those of high fat-content, to the development of unpleasant flavours through contact with ozone (page 154).

Action of Enzymes

Animal and vegetable fats, as they occur in the tissues, are invariably accompanied by enzymes capable of hydrolysing them.* These lipases vary somewhat in properties with the source from which they are derived, but all are capable of decomposing neutral fats into free fatty acids and glycerol, and all are inactivated by heat. In the living organism the enzyme is held in check, probably in the form of an inactive zymogen, and fat in the storage-depots is almost completely neutral. That from organs, such as the liver and pancreas, in which the metabolic rate is high, may, however, contain appreciable amounts of free fatty acids. When death ensues the co-ordinating mechanisms of the cell break down, and lipase begins to attack the fat. The rate of hydrolysis by the lipases of the tissues is usually small at low temperatures, and under more favourable conditions their effect is frequently overshadowed by that of the lipolytic enzymes produced by bacteria. In only a few cases

* Oxidising enzymes are considered in another section (page 52).

is spoilage by the lipases of the tissues of direct importance in the storage of food.

Fish, whose body temperatures never rise more than a few degrees above that of their environment, require for their metabolic processes powerful enzymes which retain an unusual degree of activity at low temperatures. Thus, even in the neighbourhood of the freezing point, appreciable hydrolysis of the fat of fish occurs within a few hours of death. This fact is of practical importance in the extraction of cod-liver oil for medicinal purposes, where an oil of low acid value is required. Storage of the livers overnight prior to steaming, even if the temperature is low, may result in an oil of undesirably high acidity[4]. In the case of the salmon, hydrolysis of the body-oil has been detected within a few minutes of the death of the fish[2].

The fat of chickens also appears to contain a lipase which produces a fairly rapid increase in the acidity of the fat after death[17]. Acid values* of the abdominal fats of freshly killed birds were very low (0·2), but after 24 hours at 0° C. had risen to 0·5. Birds, hard frozen for periods of from 16 months to over 7 years, showed acid values ranging up to 40. Similar data given for temperatures of 0° C. and above, fail to separate the action of the lipase of the tissue from that of micro-organisms. Pennington[17] concluded from her results that the fat-splitting enzyme present in the adipose tissue of chickens increases in activity for a time after death, owing to its gradual liberation from the inactive form (zymogen) in which it occurs in the living tissue. Activation of the zymogen present in milk is said to be caused by oxidised fats, as well as by various organic acids[6].

TABLE 18.—*Changes in free-acid content of the fat of frozen lamb.* (Lea[10])

Temperature (° C.)	Sample.	Free acidity (as % oleic acid).			
		Freshly* killed.	Increase during 7 months' storage.	Increase during 3 days' thawing.	Final value.
−20	Kidney	0·12	0·09	0·38	0·59
	Breast	0·09	0·10	0·35	0·54
−10	Kidney	0·10	0·20	0·12	0·42
	Breast	0·07	0·36	0·11	0·54
− 5	Kidney	0·14	0·64	0·14	0·92
	Breast	0·11	0·80	0·05	0·96

* The average increases in acidity of the fat of 12 carcases allowed to precool at 19° C. for 24 hours after dressing were 0·22 and 0·06 per cent. for the kidney and breast fats respectively.

* To convert acid values to per cent. free fatty acid (calculated as oleic) multiply by 0·5031.

Lea's experiments on frozen lamb (Table 18) and bacon[11] indicate that, in the fats of these meats, enzymic action is very slow at −10° and −20° C., but rather less so at −5° C. Results obtained

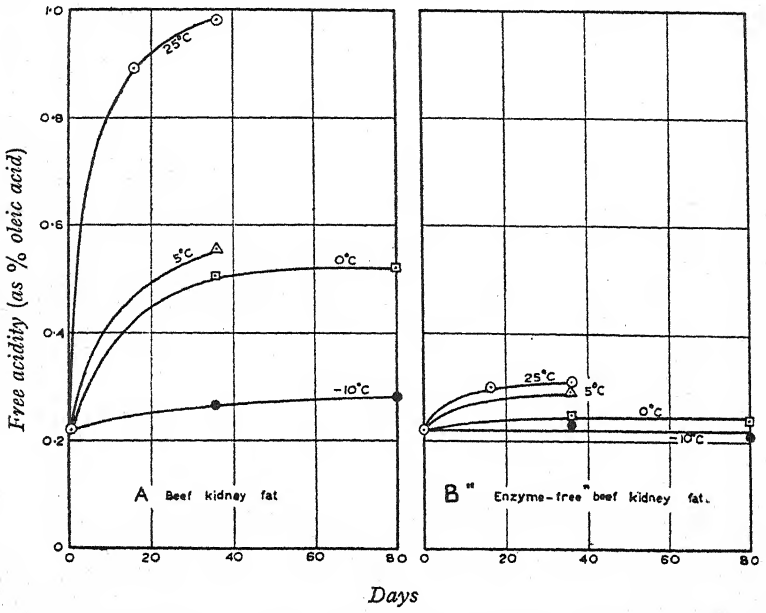

FIG. 4.—*Hydrolysis of beef-fat by the lipase of the tissue. (A) Kidney-fat under nitrogen. (B) Kidney-fat heated in vacuo at* 100° *C. for* 6 *hours and the water lost* (2·97%) *replaced. Stored under nitrogen.* (Lea[12]).

at temperatures above 0° C. (Fig. 4) in the presence of toluene probably underestimate the activity of the enzyme, which may be partially inactivated by antiseptics of this type.

Vegetable fats extracted after prolonged storage of the fruit or seed possess high free-acid contents; figures exceeding 50 per cent. have been recorded in the case of palm- and olive-oils, probably as a result of the combined action of tissue and microbial lipases. Oil-cakes also tend to develop acidity during storage, but only coconut and palm-kernel cake become rancid from this cause.

The Influence of Free Acidity on Flavour

It has long been the custom to associate deterioration in oils and fats with a high free-acid content, and the acid value has been, and is still, very widely used in the specification of products of edible quality. The usefulness of this characteristic in grading undoubtedly depends in part on the fact that fats which have been exposed to conditions leading to the production of considerable quantities of free acid are usually also rancid. The converse, however, is by no means universally true, in that fats which have become rancid through atmospheric oxidation may yet fail to show abnormally

high acidities. Recent work[1] has shown that free fatty acids prepared with adequate precautions against oxidative decomposition can be incorporated into neutral fats at concentrations up to 15 per cent. without production of any unpleasant flavour. When tasted cold, a sensation of the formation of a film on the tongue and palate was experienced with samples of the harder fats containing even 1 per cent. of free acid, but this did not increase with increasing acidity and was much less noticeable when the fat was taken in conjunction with other food. Lewkowitsch[13] states that *completely* neutral oils or fats possess an insipid taste, which is actually improved by the presence of very small amounts of free acid. These observations, however, apply only to fats which contain no appreciable quantities of acids of fewer than 14 carbon atoms, a group which includes the majority of the natural oils and fats. The case is different when, as in the milk-fats and vegetable fats of the coconut-palm kernel class, considerable proportions of volatile acids are present. These acids (C_{10}, C_8, C_6 and C_4) possess an unpleasant rancid odour and taste, and when liberated from their glycerides by the action of lipase confer these properties on the fat. Fats of this type can thus become unpalatable simply by the action of lipase.

Even in the absence of volatile acids it is usually desirable that the acid value of an edible fat should be low. The temperature at which acrid vapours are evolved, when a fat is used for frying decreases very rapidly at first with increase in free-acid content[8], a small amount of free acid producing a very appreciable reduction of smoking temperature.* The presence of free fatty acids also increases the rate of corrosion and darkening when cooking fats are heated in iron vessels.

The Measurement of Lipolytic Activity

Conflicting reports regarding the occurrence of lipase in milk and other foods appear to have been due largely to unsatisfactory methods of estimation. Many investigators, for example, measured activity in decomposing esters or glycerides of lower fatty acids, such as ethyl or amyl acetate or mono- or tri-butyrin. Methods of this kind are unreliable, since the power of an enzyme in splitting such substances may bear little relationship to its activity in decomposing glycerides of the higher acids which largely make up the natural fats. Thus, Willstatter and Memmen[23] observed that, while a preparation of pancreas hydrolysed esters of the lower acids and glycerides of the higher acids with almost equal facility, a preparation of liver was very active in hydrolysing the esters, but very slow in decomposing the glycerides. Experimental data obtained showed that 0·01 g. of the preparation of pancreas

* Rancid (oxidised) fats and fresh, neutral fats of low saponification-value (e.g., palm-kernel oil and butter-fat) also smoke at relatively low temperatures. Neutral, hydrogenated fats have high smoking temperatures.

was equivalent to 0·004 g., 1·0 g. and 106 g. of the preparation of liver for the hydrolysis of methyl butyrate, tributyrin and olive-oil respectively. A more recently reported example is the lipase of *Streptococcus lactis*, which hydrolyses tripropionin and tributyrin, but fails to attack cottonseed-oil or butter-fat[14].

In measuring the activity of a lipase some substance capable of inhibiting bacterial growth must also be present. Chloroform, acetone, iodoform and similar preservatives, which were used in the earlier work, have been found appreciably to retard the action of lipase[16]. The method of Rice and Markley[19], which utilises the preservative properties of a concentrated solution of sugar, appears to be less open to objection on this ground. The technique, as used by Nair[15] for investigation of the lipolytic activity of milk and milk-products, is carried out as follows:—

Sucrose (1500 g.) is added to raw cream containing 40–50 per cent. of butter-fat (1000 g.), and the mixture boiled for five minutes to destroy all enzymes and to facilitate solution of the sugar. After cooling to 37° C.,

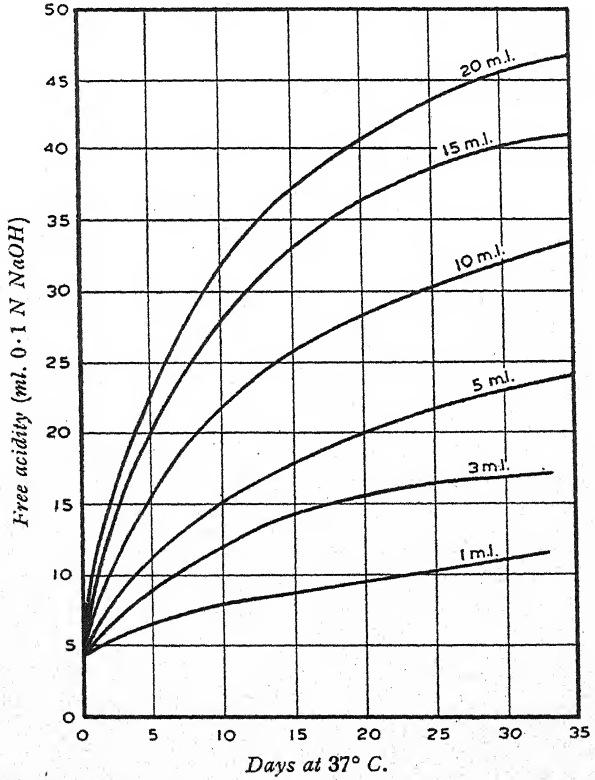

FIG. 5.—*Hydrolysis of milk-fat by the lipase of raw milk, as measured by the production of water-soluble acids.* 1–20 *ml. of milk incubated with* 100 *g. of cream-sucrose mixture.* (Nair[15])

100 g. portions of the mixture are transferred to 250 ml. Erlenmeyer flasks, which are closed with plugs of absorbent cotton. The milk (or other solution) to be tested is then added, and after thorough mixing the initial acidity is determined. The flasks are then incubated at 37° C. for three to 30 days. At suitable intervals, 10 g. samples are removed, diluted with 50 ml. of warm distilled water, and the liberated water-soluble acids titrated to phenol phthalein with 0·1 N. sodium hydroxide. Control estimations are carried out, using the same quantity of boiled solutions of the enzyme.

It has been demonstrated conclusively by this method that raw milk contains an enzyme capable of splitting the fat (Fig. 5), and hence of producing rancid flavours in milk and milk-products. Further refinements in the method seem possible for quantitative work. The water-soluble acids (C_4–C_{10}) of butter-fat account only for some 8 per cent. by weight of the total acids, so that a considerable loss in sensitivity must be entailed by titrating only this fraction of the acids liberated by the action of lipase. This difficulty can be avoided by titrating in the presence of hot alcohol, or of ether and barium or calcium chloride[3]. For investigation of the action of lipase on fat other than that of milk, the substrate should preferably consist of the fat emulsified in water to give particles of small size and approximately uniform diameter[5]. This precaution is necessary, since the rate of hydrolysis by lipase is proportional to the area of surface exposed to its action.

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PART IV.—THE ACTION OF MICRO-ORGANISMS ON FATS

In addition to a source of carbon, moulds, yeasts and bacteria require moisture, nitrogenous substances and mineral salts for their metabolism. These latter are absent from a pure dry fat, which cannot alone support growth. Micro-organisms inoculated into such a medium therefore fail to produce any chemical change, and the majority soon die. Organisms and spores of some types, however, remain dormant but viable for long periods.

Spoilage by micro-organisms thus occurs most readily in fats in the tissues, and in the great variety of prepared foods containing fat. The refined oils and fats of commerce are usually almost sterile, some giving counts as low as 10 organisms per gram[40]. Though very poor media, they usually contain sufficient non-fatty impurities to support some growth if stored in the presence of water or at very high atmospheric humidities. Commercial lard containing 0·3 per cent. of moisture inoculated with various organisms showed growth in four weeks at 37° C., whereas dehydrated lard did not[40].

Determination of the bacterial count on fat is a matter of some difficulty, owing to the immiscibility of fat with an aqueous medium. The sample to be examined should be emulsified by shaking with about 100 volumes of 0·1 per cent. sodium taurocholate or sodium oleate solution until the fat-globules are less than 10μ in diameter. Even at this state of subdivision the number of colonies on the plates will vary to some extent with the fineness of the emulsion.

Organisms which attack fatty foods are, in the great majority of cases, of non-pathological types, but they cause much damage by the production of unpleasant flavours and odours and by the formation of unsightly growths and discolorations. Moulds possess great powers of adaptation, and are capable of growth under a great variety of conditions. They flourish in quite acid solutions, and are on the whole less inhibited by low humidities and by high concentrations of sugar and salt than are bacteria. At a neutral or an alkaline pH and in the presence of ample supplies of water and nutrient, they may be overgrown and suppressed by rapidly proliferating yeasts and bacteria. Foods rich in sugar are particularly liable to attack by yeasts, which are also able to grow in moderately salt or acid solution and at lower moisture-contents than the majority of bacteria. Bacteria of various types attack any kind of food material, but the majority are inhibited fairly easily by acid pH, high osmotic pressure of the medium, and low temperature.

Relatively little is known concerning the action of micro-organisms on fats, and the following account gives only a general indication of the nature of the changes involved.

The Production of Free Fatty Acids

It is over 50 years since the production of lipolytic enzymes by bacteria growing on artificial media was first discovered, and

numerous species of moulds, yeasts and bacteria are now known to be capable of hydrolysing fats. Among fat-splitting bacteria are *Staphylococcus aureus, Staph. pyogenes albus, B. pyocyaneus, B. prodigiosus, B. choleræ, B. typhosus, Streptococcus hemolyticus, B. tuberculosis, B. lipolyticum, Micrococcus tetragenus, B. proteus, B. putrificus, B. punctatum, B. coli, Clostridium botulinum,* and various species of *Pseudomonas* and *Achromobacter.* Many of the moulds, including species of *Aspergillus, Penicillium, Mucor, Rhizopus, Monilia, Oidium* and *Cladosporium,* and numerous yeasts, also hydrolyse fat.

In some of these cases the lipoclastic enzyme remains confined within the cell, but more usually it diffuses into the surrounding

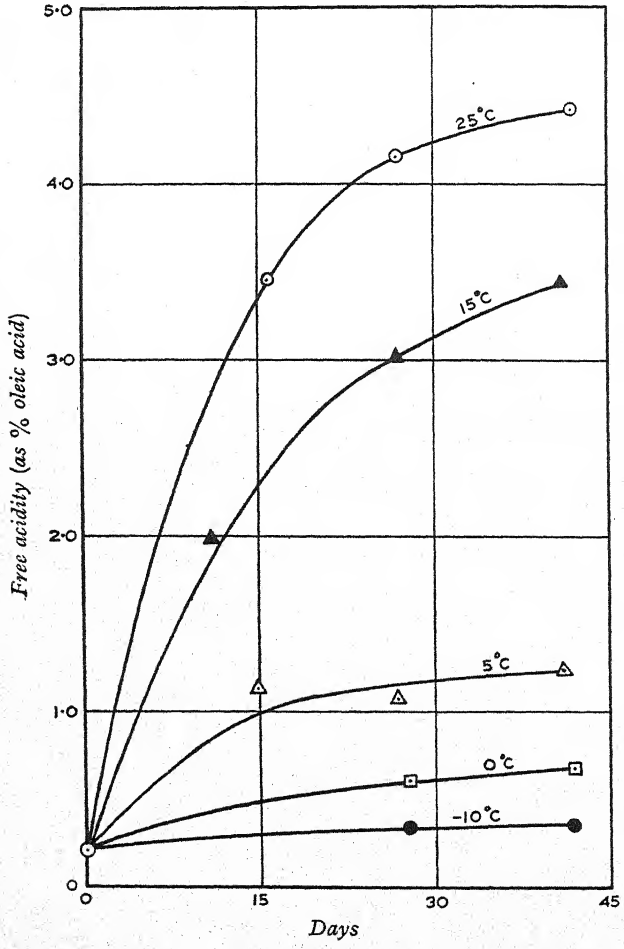


Fig. 6. *The hydrolysis of beef-fat by anaerobic micro-organisms.* (Lea[47])

medium and there produces extensive decomposition. Microbial lipases from different sources are probably not identical, since they appear to vary to some extent in stability to heat, and in the *p*H required for optimal activity. Hydrolysis of fat can be produced under both aerobic (Fig. 7) and anaerobic conditions. Fig. 6 illustrates the decomposition of beef-kidney fat in pure nitrogen by organisms acquired during the dressing of the carcase.

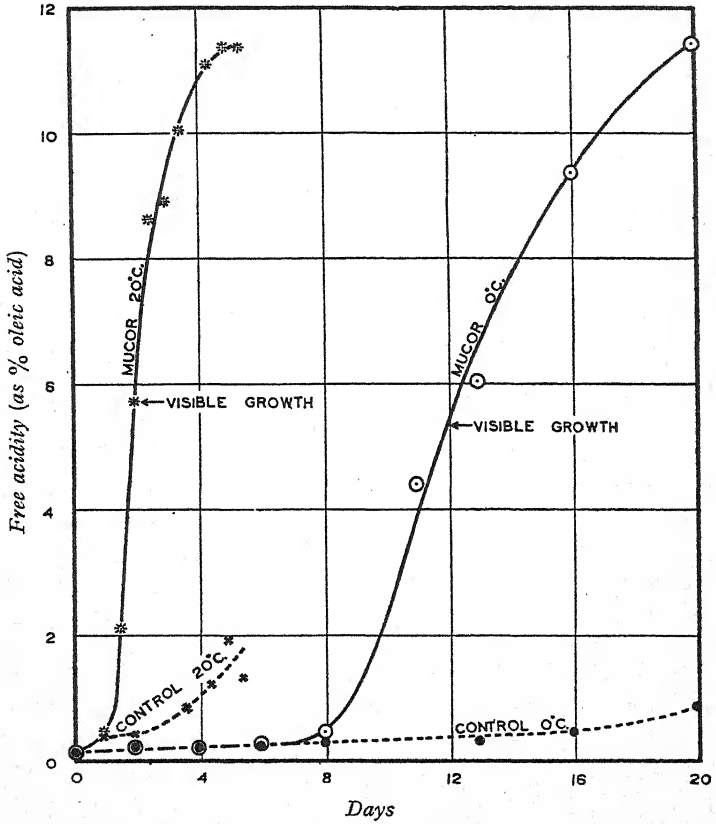


FIG. 7.—*The hydrolysis of beef-fat by Mucor at* 20° *and* 0° *C.* (Lea[46])

As already pointed out (page 44), hydrolysis of fats which contain appreciable quantities of volatile fatty acids is in itself sufficient to produce a rancid condition, and spoilage of products containing such fat readily results from contamination with powerfully lipolytic organisms. Butter, for example, may become rancid through the action of *Oidium lactis* or *Cladosporium butyri*, or of lipolytic strains of *Pseudomonas* or *Achromobacter*, or of yeasts. The majority of food-fats, however, contain no volatile acids, and hydrolysis by microbial action has little direct influence on flavour.

On the other hand, many micro-organisms produce enzymes which decompose the proteins of fat-containing foods, with the production of substances of unpleasant taste and odour, such as indole, skatole, hydrogen sulphide, methylamines and ammonia, or which break down proteins, carbohydrates and fats to propionic, butyric, lactic and other volatile fatty acids. Such organisms are responsible for " surface taint " in butter, and for tainted and sour flavours in the fat of chilled beef.

It frequently happens that a micro-organism which breaks down protein also hydrolyses fat, as found by Collins[10] for 159 cultures of bacteria studied. On the other hand, some organisms produce proteases but no lipase, while others possess pronounced lipoclastic properties but have less effect on protein. Many of the common moulds for example, hydrolyse fat with great rapidity (Fig. 7), while some strains of *Pseudomonas* and *Achromobacter*, equally capable of producing taint, are only feebly active. Obviously the free-acid content of the fat cannot be expected always to increase with the decomposition of protein which results in tainting. Nevertheless, with a mixed infection, such as is usually present in foods, determination of the acid value of the fat sometimes gives a useful indication of the progress of spoilage. The method is simple, and in suitable cases sufficiently sensitive to detect the progress of deterioration before " off " flavour or visible growth appears. Lea has found changes in free acidity of value in investigating the effect of conditions of storage, such as humidity (Fig. 32), presence of carbon dioxide (Fig. 10), and growth of mould (Fig. 7), on the development of taint. In all of these cases comparison was made between samples which carried the same initial load of (usually mixed) organisms, and in every case the free-acid values were calibrated against taste and odour. Naturally the effect of external conditions on the various organisms present was not uniform, but in the instances quoted this did not obscure the main result, and the curves obtained gave considerably more information regarding the progress of deterioration than could have been obtained from tasting tests alone.*

" *Soapiness* " *in Edible Fats*

The unpleasant soapy flavour sometimes observed in foods of high fat-content has been attributed to the formation of ammonium soaps by combination of free fatty acids with ammonia derived from protein. Alkali carbonates, resulting from microbial oxidation of

* The use of free acidity as an index of microbial spoilage in the fat of chilled meat has been criticised on the grounds that only about half of a number of strains of *Achromobacter* and *Pseudomonas* grown in pure culture on fat produced hydrolysis [68]. All of the yeasts examined hydrolysed, as well as tainted, fat, and the same is certainly true of a number of the meat-attacking moulds. Free acidity should not be used unsupported as a measure of tainting, but, used with recognition of its limitations, can prove of considerable value.

salts of organic acids, would presumably give similar results. Moulds (*Monilia*) and pink yeasts (*Torulæ*) can produce this type of spoilage in margarine[15]. For detection of the presence of such organisms a medium unfavourable to bacterial growth, e.g., wort-agar acidified with lactic or tartaric acid to *p*H 3·3–3·6 has been recommended, the plates being incubated for five days at room temperature, or two days at 30° C., followed by three days at room temperature[15]. It is possible that a soapy flavour might arise in some cases from the joint action of a powerfully lipolytic and an alkali-producing (proteolytic) organism.

Methods for Detecting the Activity of Lipase

Numerous methods have been proposed whereby micro-organisms which give rise to lipolytic enzymes can be recognised. These consist essentially in incubating the suspected organism in a nutrient medium containing fat, production of the enzyme being shown (1) by a clearing of the film of solid fat[20] or opalescent emulsion[7], (2) by the change in colour of an indicator incorporated in the medium, (3) by formation of blue-green soaps on the addition of copper sulphate, or (4) by the effect of the filtered, sterile solution in laking red blood-corpuscles[21].

Turner[67] has found that a Nile blue sulphate-cottonseed oil-agar medium, in which fatty acids produced by the action of lipase stain blue, gives a high degree of sensitivity, but that the growth of some organisms is inhibited by the medium. Jensen and Grettie[40] consider that this effect is due to the presence of fat peroxides in the oil, cottonseed and similar oils being known to reach relatively high peroxide-contents before becoming perceptibly rancid. Peroxide oxygen powerfully inhibits the growth of many species of micro-organisms, particularly reducing bacteria and fungi, which are more susceptible in this respect than organisms which themselves produce an oxidising potential. Freshly refined cottonseed oil contains no peroxide, but produces it comparatively rapidly, even during the induction-period (Fig. 33). Jensen and Grettie therefore make use of coconut and palm oils, which are more resistant to the formation of peroxide. Media suitable for detecting the activity of lipase are prepared according to these authors as follows:

Agar-stock.—0·5 g. Sodium phosphate (Na_2HPO_4) dissolved in 100 ml. of nutrient agar and the *p*H adjusted to 7.4.

Oil-emulsion.—To 200 ml. of hot distilled water are added 100 ml. of refined coconut (or palm) oil, 2 g. gum-tragacanth and 4 g. sodium taurocholate. The mixture is then shaken until the globules are of the order of 10μ in diameter, and autoclaved at a pressure of 15 lb. per sq. in. for 15 mins.

To 5·5 ml. of melted agar-stock are added 0·75 ml. of oil-emulsion and 0·75 ml. of 0·1 per cent. aqueous Nile blue sulphate. Portions of this mixture are then cooled to 42° C., the inocula at various dilutions added, and the plates poured in thin layers. Duplicate plates are prepared for each dilution and incubated at 20° C. and 37° C., respectively, for four days. Lipase-forming colonies appear blue or bluish red in colour. Counts are made in the usual manner, the number of lipoclastic bacteria per gram of fat being calculated from plates showing 30–300 colonies.

Pure triglycerides and fatty acids dispersed in Nile blue sulphate-agar show marked differences in behaviour towards the dye[10,11]. Tripropionin (C_3), tricaproin (C_6), tricaprylin (C_8) and triolein (C_{18} unsaturated) stain bright red. Tricaprin (C_{10}), trilaurin (C_{12}), trimyristin (C_{14}), tripalmitin (C_{16}) and tristearin (C_{18}) stain to a degree which decreases rapidly with increasing molecular weight, tripalmitin and tristearin being only very slightly affected. Beef-tallow, coconut-oil, corn-oil, cottonseed-oil and lard all appear bright red, but linseed- and olive-oils are rather less deep. Caproic, caprylic and oleic acids stain strongly blue, capric and lauric less so, while myristic, palmitic and stearic acids absorb very little colour. Bacteria appear to hydrolyse simple triglycerides of the saturated acids with increasing difficulty as the molecular weight increases, tristearin being completely resistant. Unsaturated triglycerides and all the common fats, however, are readily attacked, and any of the latter can be used to demonstrate the action of lipolytic bacteria. Of a large number of bacteria tested by the Nile blue sulphate technique, *Pseudomonas fragi*, *Pseudomonas fluorescens*, *Pseudomonas mucidolens*, *Achromobacter lipolyticum* and *Achromobacter conii* were the most active in producing hydrolytic rancidity in butter[10,38].

An alternative method for determination of the lipolytic powers of micro-organisms consists in titration of the free acids produced in a fat-containing medium, which may be prepared to correspond approximately in composition to that of the foodstuff under investigation. For butter-fat, for example, a medium containing cream is suitable. For investigation of the action of micro-organisms on the fat of chilled beef, an emulsion prepared from nutrient agar (25 ml.), phosphate buffer at *p*H 6·5 (20 ml.) and beef-fat (200 ml.) has been used. By this means four out of eight strains of *Pseudomonas*, four out of nine strains of *Achromobacter* and four out of four yeasts were found to be capable of hydrolysing beef-fat at −1° C.[68]

The Influence of Tissue and Microbial Oxidases on Rancidity

Deterioration of a fat through atmospheric oxidation, as will be described in Part V, is primarily due to the combination of the unsaturated constituents of the fat with oxygen to form peroxides. These then decompose into secondary products, some of which are responsible for the characteristic "tallowy" odour and flavour of oxidised fats. The fact that the reaction proceeds in the heat-treated, sterile material and in the absence of appreciable amounts of water, indicates that the participation of biological agencies is not essential to the process. Nevertheless, evidence has recently been accumulating that biological oxidising systems, either present initially or elaborated by invading micro-organisms, can greatly increase the rate of development of oxidative rancidity in fat in the tissues.

Oxidase, peroxidase and catalase have been shown to be present in the abdominal fat of freshly killed chickens[36], and peroxidase

and catalase[70] occur in raw milk. No connection, however, appears to have been established between the presence of such enzymes and keeping properties. Pure peroxidase, isolated from horse-raddish, has been incorporated in large quantities into butter without sensibly accelerating deterioration[53], and the quantity of catalase present in butter likewise seems to be without effect[3].

On the other hand, milk (page 204) has been found to contain an enzyme, " oleinase," which produces oxidative (tallowy) spoilage[41], and the same is true of the soya bean and of various other plant tissues[2, 37]. Herring's muscle contains a heat-labile system which accelerates the production of rancidity in the fat of frozen herrings (page 222), and the muscular and adipose tissues of pork and probably of other meats contain similar enzymes (page 216). Various micro-organisms[37], in particular certain bacteria which elaborate an indophenol oxidase[40], have been shown to accelerate the production of rancidity in fats, but it has not been established that the system involved is identical with the indophenol oxidase. The increased rate of formation of peroxides and aldehydes observed when fat oxidises in the presence of tissue or microbial lipoxidase, indicates that the accelerated autoxidation probably follows the normal course.

Micro-organisms which give rise to oxidising conditions, as shown by oxidation of the indophenol and similar reagents, can be identified as follows :

The material to be tested is inoculated into the fat emulsion-agar medium, as previously described (page 51), except that the Nile blue sulphate solution is omitted. Plates are poured and incubated in the normal manner. Organisms producing lipase are now charactised by a transparent or translucent zone surrounding the colony. To detect oxidising enzymes a 0·5 per cent. aqueous solution of dimethyl-p-phenylenediamine hydrochloride is poured over the surface of the plate, when colonies producing these enzymes assume a rose-red colour. A 0·4 per cent. aqueous solution of tetramethyl-p-phenylenediamine hydrochloride may be used with some gain in sensitivity, the oxidase-producing colonies being coloured in this case a deep purple. Alternatively, a mixture of 2·5 ml. of 0·14 per cent α-naphthol, 2·5 ml. of 0·17 per cent dimethyl-p-phenylene-diamine hydrochloride and 5 ml. of 0·1 per cent. sodium carbonate gives the deep violet-blue colour of indophenol blue. All of these reactions depend on oxidation of the reagent in the presence of the oxidase to a highly coloured quinonoid compound.

Jensen and Grettie[40] describe experiments in which leaf-lard and hydrogenated cottonseed-oil shortening were inoculated with cultures of organisms producing (*a*) lipase, (*b*) oxidase and (*c*) both enzymes together, and incubated at 37° C. for 14 days. The fats were then examined for (1) free acidity (hydrolysis); (2) peroxide (oxidation); (3) Kreis test (oxidation); (4) aldehydes, by the Schiff and Issoglio methods (oxidation); (5) absorption of oxygen (susceptibility to oxidation); and (6) odour and flavour—with the results given in Table 19. From these and similar data it was concluded that organisms which produce oxidases, and particularly those which also produce lipase, are potential causes of rancidity in fats.

TABLE 19.—*The effect of micro-organisms in pure culture on fat* (Jensen and Grettie [40]).*

Test	Lipase former No. 3		Oxidase former No. 48		Lipase-oxidase former No. 14		Sterile control	
	Lard	Shortening	Lard	Shortening	Lard	Shortening	Lard	Shortening
Free acidity	++	++	–	–	++	++	–	–
Peroxide	–	–	++	++	++	++	–	+
Kreis	±	±	+	+	+	+	–	–
Aldehydes	–	–	+	+	++	++	–	–
Oxygen-absorption ..	±	+	++	++	++	++	±	±
Organoleptic	–	–	± (tallowy)	± (tallowy)	++	++	–	–

* ++ = strongly positive; + = positive; ± = doubtful; – = negative.

The Reducing Properties of Micro-organisms

Many organisms by their metabolism set up reducing conditions which can readily be demonstrated by the reduction of methylene blue and similar dyes to the colourless leuco form. The more usual effect of the proliferation of a mixed flora on a fatty medium is therefore an *inhibition* of oxidation of the fat, the formation of peroxide being prevented, and even preformed peroxides being partially or completely reduced. This effect may be due either to the elaboration of reducing substances and enzymes, or simply to the potential of the free oxygen in the system being kept sufficiently low by the biological demand of the organisms to prevent oxidation of the fat.

In some cases the inhibiting effect of micro-organisms on oxidation is of practical importance. Cheeses, even when the method of preparation has been such that they contain up to 15 or 20 parts of copper per million, rarely show signs of oxidative spoilage of the fat, except perhaps at the extreme edge of the mass, where there is free access of oxygen and where desiccation may have restricted microbial activity. It can easily be shown by the incorporation of dyestuff oxidation-reduction indicators in the cheese, or by direct electrometric measurement, that the oxidising potential in the interior of the mass is maintained by the ripening organisms at values far below that at which oxidation of the fat becomes possible[13]. The same is true in the case of milk where "oily" (oxidised) flavours do not develop at ordinary temperatures in samples of high bacterial content, but may do so in low-count or pasteurized milk, particularly at temperatures sufficiently low to prevent rapid growth of the few organisms present. A similar correlation between proliferation of micro-organisms and resistance to oxidation has been shown in case of the fat of bacon (Fig. 32). It is possible that the excellent keeping properties of certain types of cured ham may be connected with the fact that during a long process of maturation the exposed surfaces become covered with a heavy superficial growth of mould.

It is not intended to suggest by these examples that microbial growth *invariably* inhibits oxidation. Obviously the precise effect obtained will depend on, among other factors, the susceptibility of the fat to oxidation, on the freedom of access of oxygen, and on the numbers and types of the organisms present.

The Decomposition of Fats and Fatty Acids by Micro-organisms

Fat is not normally utilised so readily by micro-organisms as are carbohydrate and protein. Nevertheless, many moulds, yeasts and bacteria are able to satisfy their requirements of carbon and energy from this source. Numerous organisms have been successfully grown on artificial media containing only fat or fatty acid and mineral salts, the latter, of course, including an ammonium salt or a nitrate as a source of nitrogen.

Probably all organisms which metabolise fat produce lipase, so that one and perhaps the first stage in the process consists in decomposition of the glycerides into glycerol and free fatty acids. Glycerol, which is water-soluble and akin structurally to the sugars, is readily utilised as fuel, and burned (ultimately) to carbon dioxide and water. Not less than 20 other compounds, mainly aliphatic alcohols, aldehydes and acids, have been reported as produced by the action of micro-organisms on glycerol[8]. The methods by which the free fatty acids or their salts are decomposed, are analogous to the processes of fat-metabolism in the higher animals, the net result in both cases being that the long carbon chain is gradually broken down and the fragments oxidised to carbon dioxide and water.

$$R.CH_2CH_2.COOH + O_2 \longrightarrow R.CO.CH_2.COOH + H_2O$$
$$R.CO.CH_2.COOH + H_2O \longrightarrow R.COOH + CH_3.COOH$$
$$CH_3.COOH + 2O_2 \longrightarrow 2CO_2 + 2H_2O$$

In the process of β-oxidation illustrated in the equations, the fatty-acid chain loses two carbon atoms at each step. This is probably the most important, but it is certainly not the only route by which the biological oxidation of fatty acids can occur in the living organism (page 83). Acids of lower molecular weight, formed as intermediate products, do not usually accumulate in any appreciable quantity, probably because they are themselves oxidised even more readily than the original substance.

Organisms growing under anaerobic conditions on media containing fatty acids convert the latter almost quantitatively into carbon dioxide and methane according to the general equation :

$$4\,C_nH_{2n}O_2 + 2(n-2)H_2O \longrightarrow (n+2)CO_2 + (3n-2)CH_4$$

where n is the number of carbon atoms in the acid[51]. The presence of a trace of hydrogen and a little excess of carbon dioxide in the gases evolved is presumably due to a side reaction of the type :

$$CH_3.COOH + 2H_2O \longrightarrow 2CO_2 + 4H_2$$

In both of these cases water without oxygen has been used to break down the chain and oxidise part of it to carbon dioxide. It is typical of anaerobic metabolic processes in general that the energy liberated by complete decomposition of a gram molecule of fatty acid in this way is only a very small fraction of that liberated by the ordinary aerobic oxidative process.

Ketone Rancidity

The occurrence in foods containing certain fats, notably coconut and palm-kernel oils and butter-fat, of a peculiar type of rancidity characterised by a distinctive but not always unpleasant " ester-like " odour and a pungent, unpleasant taste, was recognized under various names for many years before the mechanism of its production was understood. Haller and Lassieur[35] had identified in the strongly smelling oil recovered during the commercial deodorisation of crude coconut-oil with steam, methyl heptyl and methyl nonyl

ketones, together with smaller quantities of methyl undecyl ketone and of methyl heptyl and methyl nonyl carbinols. These must have been produced during drying of the copra or during storage of the oil, since coconut-oil expressed from the fresh nut has a mild, agreeable, almond-like odour and taste.

Stokoe[62] in 1921, however, appears first definitely to have attributed "perfume" rancidity in coconut-oil and in butter and margarine to the presence of ketones rather than of aldehydes or esters, and to have shown that these are produced by the agency of a particular group of micro-organisms. Later investigators[61, 16] of this defect, particularly in coconut-oil, have confirmed the finding that the characteristic odour developed is due to the action of moulds, mainly *Penicillium* and *Aspergillus*, on the fat in the presence of moisture and nitrogenous material[1, 63]. Spoilage thus occurs most readily in products such as copra (coconut) prior to extraction of the oil, in coconut and palm-kernel press-cakes, and in margarine prepared by churning these fats with milk. Butter and other foods containing milk-fat together with moisture and nutritive material may also develop ketone rancidity. The odour itself appears to be due predominantly to ketones, particularly the methyl amyl, methyl heptyl and methyl nonyl compounds. Smaller quantities of methyl propyl and methyl undecyl ketones, however, are usually also present, together with methyl heptyl and methyl nonyl carbinols and free volatile acids and esters[63].

The production of ketones has been observed with nine *Penicillia*, five *Aspergilli*, *Cladosporium herbarum*, *Cladosporium butyri*, and several other moulds growing on inorganic media with coconut-oil as the sole source of carbon. *Oidium lactis*, however, though growing vigorously, failed to produce ketones[16].

Mechanism of the Formation of Ketones

The chemistry of the production of ketones by moulds has received considerable attention, probably as much on account of its bearing on the problems of the metabolism of fat in general as by reason of its practical importance in the deterioration of food. In attacking the fat the organism is generally assumed first to split the glyceride molecules into free fatty acids and glycerol (though there is some lack of evidence on this point), subsequently oxidising the latter as already described.

Decomposition of the fatty acids appears to follow the general lines of the Dakin synthesis, in which small yields of methyl ketones are obtained by β-oxidation of the ammonium or sodium salts of saturated fatty acids with hydrogen peroxide (page 82). One notable difference, however, exists between the two cases. Saturated fatty acids up to and including stearic acid (at least) yield the corresponding methyl ketone on treatment with hydrogen peroxide. Moulds, on the other hand, only produce ketones from acids of medium molecular weight, and fail to do so from either the lowest or the higher members of the series. The data given in Table 20

refer to the action of *Penicillium glaucum* on individual fatty acids in artificial media containing nitrogen. The (liquid) ketones were identified in the steam distillates from the cultures by conversion to crystalline semicarbazones.

TABLE 20.—*The production of methyl ketones from fatty acids by the action of moulds.*

(Starkle[61])

Acid.	Number of carbon atoms.	Corresponding ketone.	Detected by odour.	Identified chemically.
Butyric† ..	4	Acetone	—	—
Valeric ..	5	Methyl ethyl ketone ..	—	—
Caproic* ..	6	Methyl propyl ketone ..	+	+
Heptylic ..	7	Methyl butyl ketone ..	+	+
Caprylic* ..	8	Methyl amyl ketone ..	+	+
Nonylic ..	9	Methyl hexyl ketone ..	+	+
Capric* ..	10	Methyl heptyl ketone ..	+	+
Lauric* ..	12	Methyl nonyl ketone ..	+	+
Myristic* ..	14	Methyl undecyl ketone ..	+	—
Palmitic* ..	16	Methyl tridecyl ketone ..	—	—
Stearic* ..	18	Methyl pentadecyl ketone	—	—
Oleic* ..	18	—	—	—

* Present in coconut-oil, palm-kernel oil and butter-fat.
† Present in butter-fat.

Since ketones are not produced by the action of micro-organisms on acids of higher molecular weight than myristic, and only in traces and under favourable conditions from this, the majority of the common fats of food, which contain no appreciable quantity of acids lower than myristic, do not develop ketone rancidity.

The explanation usually advanced to account for the selective formation of odorous methyl ketones only from fatty acids of medium molecular weight is based on the observation of Spieckermann[60], that the toxicity of free fatty acids towards *Penicillium glaucum* increases with molecular weight to a maximum at caprylic acid (C_8), and then falls again to a low value for the solid, high melting, insoluble fatty acids.

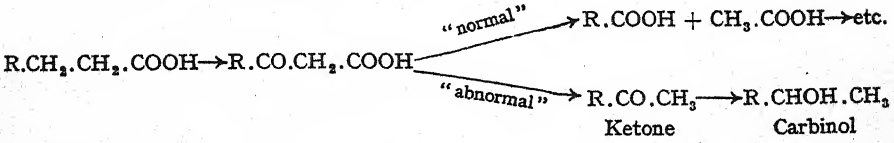
Degradation of the fatty acids in the normal healthy cell is considered to proceed by β-oxidation to the keto acid, which then splits by hydrolysis to yield an acid containing two carbon atoms less. This in turn oxidises to its β-keto acid, and thus the process continues. In a cell poisoned by toxic fatty acids, enzymic activity is impaired, and the oxidative process tends to stop short at the keto acid. This substance readily loses carbon dioxide by the action of carboxylase, and thus becomes converted to the methyl ketone, which the enfeebled mould is unable to oxidise further. Ketones therefore accumulate in the medium. It has been suggested[61] that the poison-

ing action involved consists in adsorption of the fatty acids on the mycelium of the mould, by which the normal processes of respiration and oxidation are impaired.

Certainly ketones are readily formed when the mould is growing feebly, and tend to disappear when a vigorous aerial mycellium is produced[16]. Similarly, the formation of ketones is favoured by a medium of low nutritive value, and reduced by the presence of nutrients such as sugar[16]. Butter-fat, which contains a very much smaller proportion of lower fatty acids than coconut or palm-kernel oil, gives ketones less readily than the vegetable oils, but can nevertheless be spoiled from this cause under suitable conditions. The joint presence of powerfully lipolytic organisms and formers of ketones probably favours the production of ketone rancidity.

Moulds which form ketones do not all do so with the same facility. Some produce ketones under most circumstances, but many others do so only under specially favourable conditions. A few, however, including *Oidium lactis* and *Mucor*[61], seem able to oxidise fatty acids in the normal manner under any conditions which permit growth, and cannot be made to produce ketones.

The two types of metabolism of fatty acids, "normal" and "abnormal" are illustrated below:



Formation of the β-hydroxy acid cannot be the first link in the oxidation chain, since *Penicillium glaucum* is apparently unable to produce the ketone from this substance[1]. β-keto acids *in vitro* readily lose carbon dioxide to form ketones, but β-hydroxy acids do not form carbinols in this way. The carbinols found in rancid coconut-oil therefore probably originate from the reduction of the corresponding ketones, a reaction which has been observed to occur in cultures of yeast[52].

The Chemical Detection of Ketones

Identification by odour and taste of ketone rancidity in its more advanced stages presents little difficulty, particularly in products of mild flavour. The characteristic pungent, penetrating, aromatic odour of methyl heptyl or methyl nonyl ketone, for example, can be recognised in edible fats at concentrations as low as from four to 60 parts per million, depending on the experience of the observer[57]. For detection of incipient spoilage, however, particularly in foods of pronounced flavour, and as a means of distinguishing ketone from ordinary oxidative (aldehydic) or hydrolytic rancidity, some kind of chemical test is necessary. The method most generally used is that of Täufel and Thaler[64], which is based on earlier observations[22, 14], to the effect that salicyl aldehyde, in the presence of

sulphuric acid, gives coloured condensation products with ketones containing the $-CH_2.CO.CH_2-$ group in the molecule. The actual colour developed varies with the length of the chain from orange with acetone to a deep raspberry red with methyl nonyl and higher ketones. The test is applied as follows :

From a 200 ml. glass-stoppered distilling flask, fitted with a short condenser and containing 160 ml. of water, distil over 25–30 ml. into a 50 ml. glass-stoppered tube or cylinder. To the distillate add 0·4 ml. of pure* salicyl aldehyde and shake vigorously. Centrifuge or allow to settle, and pour off the supernant aqueous fluid down to a volume of approximately 4 ml. Shake again and drop into the emulsion, without touching the walls, 2 ml. of concentrated sulphuric acid. Shake vigorously and allow to stand. The salicyl aldehyde which separates as an upper layer should in this blank test show only a pale yellow, or at most a faint pink, colour. Neither rubber nor cork must be used in the apparatus.

To the remaining water in the flask add 5 or 10 g. of the carefully melted fat by means of a long-stemmed funnel, distil over a further 25–30 ml. and test with salicyl aldehyde and sulphuric acid as before. In the presence of ketones the layer of aldehyde is coloured pink to deep red. A faint colouration can be intensified by immersing the tube for 15 mins. in a boiling water-bath.

The limit of sensitivity of the method is approximately 5×10^{-6} g. (5γ), which on 5 g. of fat corresponds to a dilution of one part per million.

Schmalfuss, Werner and Gehrke[56] have made extensive use of a modified form of the Täufel and Thaler test in which the danger of erroneous results through sulphuric acid coming into direct contact with the aldehyde is avoided :

In this method a 100 ml. flask fitted with a splash-head and condenser is used, and the blank determination carried out as described above. 10–25 g. of fat, 25 ml. of saturated sodium chloride solution and a little porous pot are then added, and 4 ml. distilled over into a stoppered tube. To this distillate are added 0·2 ml. of salicyl aldehyde and 3 ml. of fuming hydrochloric acid, the mixture being then shaken and heated to boiling. After standing for one minute (or with very small amounts of ketone three minutes), 0·5 ml. of choloroform is added and the tube shaken. In the presence of ketones the layer of chloroform which settles to the bottom is coloured red, and can be removed if desired for estimation in a tintometer. The authors claim that amounts of methyl ketones as low as 2×10^{-6} g. can be detected by this method.

Täufel and Thaler in their original paper state that fresh fats give no colour in the test, that a distinct red indicates incipient rancidity, and that a deep red is usually accompanied by an obvious rancid odour. Subsequent work[23], however, has shown that fresh butter gives a positive reaction, owing to the presence of acetyl methyl carbinol ($CH_3CO.CHOH.CH_3$). Estimations on butter-fat should therefore be carried out with the addition of ferric chloride to the distilling flask, whereby the carbinol is oxidised to diacetyl, which does not react in the test, while the ketones remain unchanged[54].

* It is essential to purify commercial salicyl aldehyde *via* the bisulphite compound.

Discoloration Produced by Micro-organisms

Micro-organisms growing in large numbers on a foodstuff can spoil its appearance simply by the presence of their colonies. Moulds are usually white at first, but many become coloured grey, black, yellow, green, greenish-blue, or orange to red, on further development. Growth may be confined to the surface, in which case the colonies can easily be removed, or the mycelia may penetrate to some depth into the medium. " Black spot " on meat and other foods for example, is due to the dark hyphae of *Cladosporium herbarum* ramifying in the tissues. Visible colonies of yeasts and bacteria may be white, yellow, brown, red or purple, and range from discrete hard nodules to a continuous slimy film.

Many organisms of all three types elaborate pigments which diffuse out of the cells and discolour relatively large volumes of the surrounding medium. Little is known of the chemical structure of these microbial pigments, but many are fat-soluble, water-insoluble substances, and some are probably carotinoids.

A number of organisms produce chromophoric substances which change in colour with *p*H. The yellow pigment of the *Staphylococci*, for example, becomes red on addition of alkali, while the yellow colour of *Sarcina flava* turns green in the presence of acid. Various moulds, bacteria and particularly yeasts produce red pigments, soluble in fat. One yeast at least produces a colourless chromogen which becomes deep red in the presence of iron salts. Proteolytic organisms which form indole and skatole, give the red colour of nitroso-indole in the presence of a nitrite, *e.g.*, on cured meats.

Jensen and Grettie[40] report a case of particular interest in which a microbial pigment functions as an oxidation-reduction indicator. Several lipolytic micrococci and bacilli, growing on fat, were found to produce a bright yellow pigment which was soluble and gradually diffused out of the cell. When the fat became rancid through oxidation, or in the presence of oxidising bacteria, the colour changed to bluish-purple. The same colour could be produced by the addition of hydrogen peroxide to a solution of the pigment. This observation probably accounts for the purple " stamping ink " discolorations sometimes found on the fat of beef and hams, the origin of which had remained somewhat mysterious, since no purple organism could be isolated from the fat.

The Control of Microbial Spoilage

Decomposition by micro-organisms can be reduced or prevented in a considerable number of ways, but only those of particular interest in the storage of the more important fat-containing foods will be considered in any detail here.

Chemical Preservatives

Small quantities of substances such as formaldehyde, benzoates, fluorides, sulphites and borates have frequently been incorporated into foodstuffs with the object of retarding or temporarily inhibiting

the growth of micro-organisms. In recent years the presence of chemical antiseptics in food-products has come to be regarded with disfavour, owing to the harmful, or at best doubtful, physiological effects produced by their continued absorption. Their use, with a few carefully controlled exceptions, has therefore been prohibited in most countries. Chemicals such as sodium hypochlorite and formaldehyde, however, still find application in the sterilisation of food-processing plant and equipment, and in keeping down the numbers of organisms in the interiors of buildings housing such plant.

Reduction of Contamination

The first and most logical method of combating microbial spoilage, as well as food-borne disease, is the reduction of contamination with undesirable organisms during preparation and handling. In recent years much progress has been made in this direction, whereby the keeping properties of many food-products have been greatly improved. The dairy industry alone provides sufficient example of the notable advance in consistent quality obtainable by this means. In many cases, however, room remains for much further improvement. To quote an example: an enormous variation is often found in the initial contamination of meat during dressing at slaughter-houses

TABLE 21.—*The relation between initial bacterial count and storage-life of meat.*

(Haines[28])

Initial bacterial count (per sq. cm.)	43	270	2,800	17,300	40,000
Days required for the appearance of "slime"* at 0°C. and 100% R.H.	18	16	11–12	10	8

* The first appearance of "slime" corresponds to a count of approximately $10^{7 \cdot 5}$ organisms per sq. cm.

of different types[24], and this factor is undoubtedly of great importance in determining the subsequent storage-life of the meat (Table 21).

The Use of Sugar and Salt

Hardly to be classed with chemical antiseptics, and yet widely used in the preservation of food, are sugar and salt. Strong solutions of sugar inhibit the growth of micro-organisms, probably owing to the resistance presented by their high osmotic pressures to the free passage of water into the cell. A few bacteria, some moulds, and particularly yeasts, however, are able to grow freely in concentrated solutions of sugar. Preservation by this method is therefore only relative, and is usually applied, in conjunction with initial sterilisation, to control decomposition by air-borne organisms.

Common salt also has a marked inhibiting action on the growth of most organisms, and in conjunction with reduction of moisture-content by drying is used for the preservation of fish, meat, nuts,

etc. Butter containing 1·0–2·0 per cent. of salt (8–16 per cent. on the aqueous phase) is much more resistant to microbial spoilage than the unsalted product. Some organisms, however, will grow even in strong (25 per cent.) brine, and spoilage of salted fish and similar foods can sometimes be traced to bacteria or yeasts introduced in the salt itself.

Low Temperatures

The range of temperatures over which micro-organisms grow and multiply is relatively limited. At first the rate of growth increases with the temperature to a maximum usually between 20° and 30°C. or at about 37°C., after which it falls away again rapidly. With the exception of a few thermophyllic bacteria and spores which resist boiling water for considerable periods, most organisms are killed by exposure to a temperature of 60°–65°C. for 30 minutes, and many much more easily. On this depend the processes of canning and bottling, whereby food-products are sterilised by heat and hermetically sealed to prevent recontamination.

TABLE 22.—*The effect of temperature on the rate of growth of bacteria.* (Haines[25])

Organism.	Source.	Number of days for visible growth at (° C.).									
		37°	20°	15°	10°	5°	0°	−1°	−2°	−3°	−5°
B. subtilis ..	—	<0·5	2	9	>30	—	—	—	—	—	—
B. coli communior ..	Abattoir	<0·5	2	2	6	*	*	—	—	—	—
B. coli communis ..	,,	<0·5	2	2	4	9	29	—	—	—	—
B. proteus ..	—	<0·5	2	3	5	24	*	—	—	—	—
S. albus ..	Chilled beef	<0·5	3	>20	—	*	—	—	—	—	—
B. lactis aerogenes ..	—	<1	2	3	9	36	*	—	—	—	—
B. mesentericus	—	<1	2	3	5	12	43	—	—	—	—
Flavobacterium	Chilled beef	<1	<1	1	2	5	>30	—	—	—	—
M. luteus ..	,,	1	2	6	23	—	>30	—	—	—	—
M. aurantiacus	,,	1	2	4	5	15	24	—	>30	—	—
Sarcina flava	Abattoir	*	2	4	5	12	75	—	—	—	—
Pseudomonas	Chilled beef	*	1	2	3	4	6	—	23	37	—
,,	,,	*	1	2	3	6	13	18	23	33	80
Achromobacter	,,	*	1	1	2	3	5	—	8	14	—
,, ,,	,,	*	1	2	3	4	16	—	41	>71	—
,, ,,	,,	*	2	2	3	4	11	13	15	26	*
,, ,,	,,	*	2	2	5	11	>30	*	*	*	*
,, ,,	,,	*	2	3	6	6	14	17	21	32	90
Yeast ..	,,	*	2	2	3	6	10	—	15	20	>40
,, ...	,,	*	2	2	4	7	11	—	16	23	>40

* Signifies no growth. Cultures at and below 0° C. were kept for three months.

From a study of the effect of low temperatures on the rate of growth of bacteria (Table 22), Haines divided the organisms studied roughly into four groups as follows :—

Group I. The *Staphylococci*, optima at 37°C., growing very slowly, if at all, below 10°C.

Group II. Most strains of *B.coli*, *B.proteus*, *B.subtilis*, etc., (optima 37°C.) and *Micrococci* (optima 20°–30°C.), growing very slowly in the range 5°–0°C.

Group III. Some strains of *B.proteus*, etc., capable of growth at 0°C.

Group IV. Most strains of *Achromobacter*, *Pseudomonas* and various yeasts, showing comparatively rapid growth at 0°C. (sometimes in five days), and growing down to about —5°C. on supercooled agar. *Achromobacter* isolated from fish have been found to grow down to —7·5°C.[4]

Several strains of *Actinomyces* isolated from commercial cold stores had, as regards temperature, characteristics similar to those of the bacteria of group II; another belonged to group IV[27].

The majority of moulds and yeasts have optima in the neighbourhood of 20°–30°C., and the growth of some species, *e.g.*, *Cladosporium herbarum*[5] and *Sporotrichum carnis*[26], has been observed as low as —7°C. This, or at most —10°C., appears to be the lower limit for the growth of micro-organisms.

The fact that microbial growth and spoilage cannot occur in hard-frozen foods does not mean that no organisms survive cold storage. Pathogenic bacteria have been found to resist temperatures of —252°C. for many hours, and living organisms are still present after months at temperatures well below zero. Even repeated freezing and thawing fails to destroy bacteria completely. Nevertheless, freezing and storage in the frozen state results in heavy

TABLE 23.—*The survival of bacteria in frozen suspension at various temperatures.*

(Haines[31])

Temperature of storage (°C.).	*Pyocyaneus.*	*B. coli communis.*	
	Organisms living after 20 days.	Organisms living after 11 days.	Organisms living after 42 days.
Before freezing ..	—	2,000,000	
After freezing ..	1,000,000	1,360,000	
—20°	300,000	417,000	367,000
—10°	100,000	314,000	110,000
—5°	160	46,000	8,000
—3°	80	57,000	800
—2°	5	17,000	170
—1°	—	91,000	1,300

mortality, the effect varying with the nature of the organism. Very rapid freezing at −70°C., for example, has no appreciable effect on *Staphylococcus aureus*, but kills 30 per cent. of *B.coli*, 60 per cent. of *Achromobacter* and 80 per cent. of *Pyocyaneus*[33]. Spores are generally more resistant to freezing than vegetative organisms. The rate of death in the frozen state of the organisms surviving freezing is greatest at temperatures between −1° and −5°C., and relatively slow at −20°C. (Table 23). Some spores, however, can be frozen at −2°C. for months without appreciable reduction in numbers.

Control of Humidity

Though some micro-organisms, and particularly spores, resist desiccation for long periods, there is usually a tendency for the microbial count to fall when the environment fails to provide sufficient water for growth.

TABLE 24.—*Minimal atmospheric humidities and water-contents of muscle permitting growth at* −1°C.

(Scott[55])

Organism.	Type.	Atmospheric humidity. (%)	Water-content of the muscle. (%*)
Achromobacter (2 strains) ..	Bacterium	96–96·5	85–90
Pseudomonas (2 strains)	„	98–98·5	140–180
Candida sp.	Yeast	91–92	50–54
Geotrichoides sp.	„	90–91	47–50
Mycotorula sp.	„	89–90	45–47
		Fresh muscle	290–330

* Calculated on the dry weight.

The amount of moisture available at the surface of a food depends on the water-content of the latter, and on the humidity and rate of movement of the air. If the water-content of the nutrient is low, growth will only be possible at very high atmospheric humidities (Fig. 8), bacteria being, on the whole, even more readily inhibited by a restricted water-supply than moulds and yeasts. In Table 24 are given recent data for the minimal atmospheric humidities at which bacteria and yeasts isolated from chilled beef are able to grow at −1°C. on thin slices of beef-muscle dried to equilibrium with the atmosphere. The corresponding water-contents of the muscle, calculated as percentage on the dry weight, are given in the last column.

If the food contains much water, the humidity of the atmosphere is of less importance. Moulds on nutrient media of high water-content, for example, grow almost as rapidly over concentrated sulphuric acid as over water[48], moisture being transferred from the interior to the surface sufficiently rapidly to replace much of

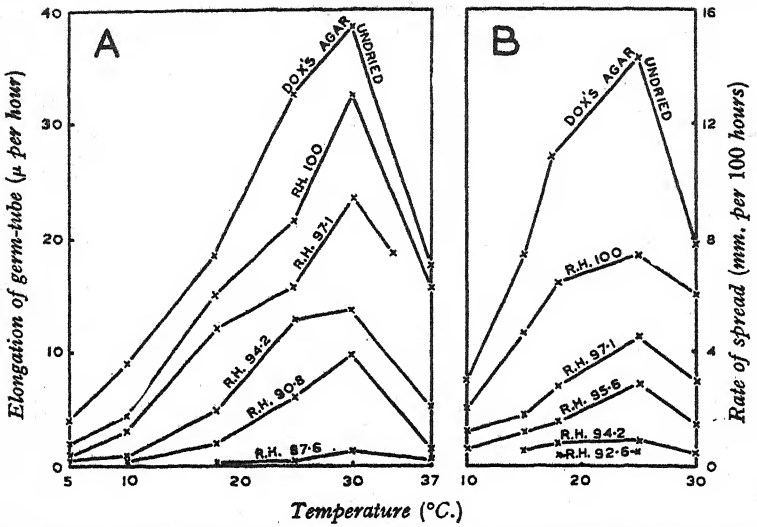


FIG. 8.—*The effect of humidity of the atmosphere on the germination and growth of moulds on anhydrous nutrient.* (*A*) *Alternaria citri*; (*B*) *Trichoderma lignorum.* (Tomkins[66])

that lost by evaporation. The film of air over the surface is therefore maintained at a much higher humidity than the general atmosphere of the vessel or chamber. Thus, it has been found that the appearance of visible mould on the surface of freshly cut muscle of meat (75 per cent. water) held in small refrigerators in still or slowly moving air, is not greatly delayed by considerably reducing the humidity of the air, though the total loss of weight is greatly increased[59].

In the large-scale storage and transport of meat most of the muscle is protected by a superficial layer of connective tissue and fat. This has only a low water-content[43], and does not readily replace moisture evaporated from its surface. Microbial growth on the adipose tissue of meat is therefore sensitive to atmospheric humidity. Fig. 32 shows the varied rates of hydrolysis (by microbial action) in the fat of bacon stored in still air at relative humidities of from 60 to 100 per cent.* Similar results have been obtained with beef-fat at 0° C., using the method of bacterial counts[29]. In this case large increases in the numbers of bacteria on the external fat took place at 100 and 90 per cent. relative humidity, but practically none at 76 per cent. On kidney-fat, which contains still less moisture, the increase was rapid at 100 per cent., considerably less so at 90 per cent. and absent at 76 per cent.

* The higher free-acid values obtained at 90 per cent., as compared with 100 per cent., relative humidity are due to the powerful lipolytic action of the moulds growing most freely under these conditions.

The humidity of the air in the space above a stored product will be determined by the composition of the material and by the degree of ventilation. For many foods critical moisture-contents have been found above which, under the usual conditions of storage, they readily develop microbial spoilage. Flour, for example, containing the usual 11 per cent. of water keeps satisfactorily; above 12 per cent. it shows a decreasing resistance to mould. Rice keeps well when 10–12 per cent. of moisture is present; at 14 per cent. deterioration is rapid. Moulds are said to multiply in cottonseed meal only when the water-content exceeds 14 per cent., while copra (coconut) containing as little as 9 per cent. of moisture is attacked.

The presence of refrigerated surfaces in a store results in the maintenance of a lower effective humidity at the expense of slow distillation of water from the stored product to condense as water or ice on the cooled surface. This process, increased in efficiency by forced air-circulation, assists in keeping down the growth of moulds and of slime-forming bacteria during the transport of chilled beef.

Marked fluctuations of temperature in stores are harmful, since air which has taken up moisture at a higher temperature may become supersaturated as the temperature falls and deposit a film of water on the surface of the stored product, thus for a time providing conditions extremely favourable for microbial growth. A similar effect occurs when frozen articles are transferred to a warmer atmosphere.

Storage in Carbon Dioxide

As long ago as 1882 Kolbe observed that carbon dioxide has a preservative action on meat, and by 1889 it had been established that the growth of many bacteria is inhibited by the gas. Subsequently Brown[6] demonstrated the marked inhibiting effect of carbon dioxide on the growth of certain moulds in pure culture,

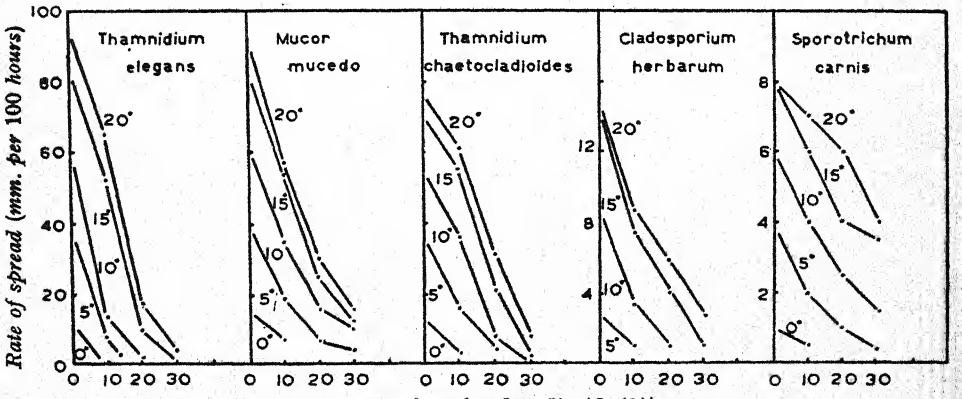


FIG. 9.—*The rates of growth of meat-attacking fungi at various temperatures and concentrations of carbon dioxide.* (Tomkins[65])

while Killefer[42] showed that the keeping properties of a wide range of foodstuffs can be appreciably improved by storage in atmospheres of this gas. Recent work at the Low Temperature Research Station, Cambridge, has greatly extended knowledge in this field, and carbon dioxide is now finding increasing application in the storage and transport of food.

Carbon dioxide is particularly effective in reducing spoilage by moulds[50, 65]. In atmospheres containing the gas the latent period of germination is greatly increased, and the percentage of spores germinating reduced. Subsequent growth is also retarded (Fig. 9), the effect varying considerably with the organism and being more marked the lower the temperature and the weaker the nutrient. The actual concentrations required completely to inhibit the growth of *Thamnidium chaetocladioides*, *T. elegans*, *Mucor mucedo* and *Cladosporium herbarum*, were 30 to 40 per cent. at 15°, 20 to 30 per cent. at 10°, 20 per cent. at 5°, and 10 to 20 per cent. at 0° C. *Sporotrichum carnis* was less affected by carbon dioxide, more than 30 per cent. of the gas being required for complete inhibition at 5° C., but the growth of this organism, even in air, is very slow. Carbon dioxide has no marked killing action on spores. Brief exposure to high concentrations of the gas is therefore of no value for the prevention of mould.

The effect of carbon dioxide on yeasts and bacteria varies from almost complete inhibition in some cases to negligible effects in others, the influence of the gas being most marked at temperatures below the optimum for growth. A few of the data which have been collected for bacteria are reproduced in Table 25. Various anaerobic organisms, including *Clostridium botulinum*, are markedly inhibited by carbon dioxide, even at 37° C.[12]

More detailed examination of the effect of atmospheres containing 10 and 20 per cent. of carbon dioxide on the growth of several bacteria isolated from meat, showed that the effect on organisms of the *Proteus* type is small, but that *Achromobacter* and *Pseudomonas*, types largely responsible for bacterial spoilage in chilled beef, are markedly inhibited, the generation-time and the time necessary for the attainment of maximal growth being approximately doubled by 10 per cent. of carbon dioxide at 0° C.[34]

As might be anticipated from these results, fat in the tissue can be successfully stored for appreciably longer periods in atmospheres containing carbon dioxide than in air[50, 45]. Naturally, the degree of protection afforded by the gas is liable to vary with the nature of the organisms present, but in several experiments with beef (back) fat the length of time required for the development of taint at 0° C. was found to be approximately doubled in the presence of 10 per cent. of carbon dioxide (Fig. 10). Appreciably greater protection was given by 20 per cent. of the gas, but, in the experiments quoted, very little further advantage was obtained by increasing the concentration beyond this point[45].

TABLE 25.—*The effect of carbon dioxide on the growth of bacteria* (Coyne[12]).

Organism	Days to attain definite growth at														
	Temperature 0° C.					Temperature 10° C.					Temperature 25° C.				
	Air	5%	10%	15%	20%	Air	10%	20%	50%	100%	Air	10%	20%	50%	100%
Achromobacter ..	5	6	6	16	>28	1	2	2	12	>40	<1	<1	1	3	9
,, ,, ..	6	8	14	14	36	1	1	2	8	>40	<1	<1	<1	3	6
,, ,, ..	4	7	8	8	14	<1	1	2	5	20	<1	<1	<1	2	3
Flavobacter ..	12	16	16	>28	>28	2	3	4	4	8	<1	<1	<1	1	2
Micrococcus ..	18	18	24	>40	>40	3	4	6	8	16	1	1	1	3	9
Pseudomonas ..	6	6	12	16	18	1	1	2	6	>40	<1	<1	<1	1	4

	Air	50%	100%	Air	50%	100%	Air	50%	100%
*Aerobacter** ..	12	>40	>40	1	2	4	<1	<1	<1
,, ..	6	20	36	1	2	4	<1	<1	<1
,, ..	>40	—	—	1	2	4	<1	<1	<1
Proteus	>40	—	—	1	2	6	<1	<1	<1
Staphylococcus aureus	—	—	—	6	>40	>40	<1	<1	2
B. coli	—	—	—	4	6	16	<1	<1	1
B. aertrycke ..	—	—	—	4	4	8	<1	<1	1
B. enteritidis gaertner ..	—	—	—	4	5	6	<1	<1	1

* The majority of the organisms which showed definite growth in less than one day at 25° C., even in 100 per cent. carbon dioxide, required times of the order of 1, 2 and 3 days to attain well developed growth in air, 50 per cent. and 100 per cent. of carbon dioxide respectively.

Pork, like beef, can be kept in good condition for much longer periods in atmospheres containing carbon dioxide than in air, though higher proportions of gas have been recommended for this purpose[9]. Atmospheres of pure nitrogen or hydrogen inhibit ærobic organisms, but permit the growth of anærobes, which rapidly spoil the meat. In the case of bacon, the superficial growths developed in air at high humidities consist of salt-tolerating organisms, mainly moulds and yeasts. These can be kept in check by complete exclusion of oxygen, which also prevents oxidative

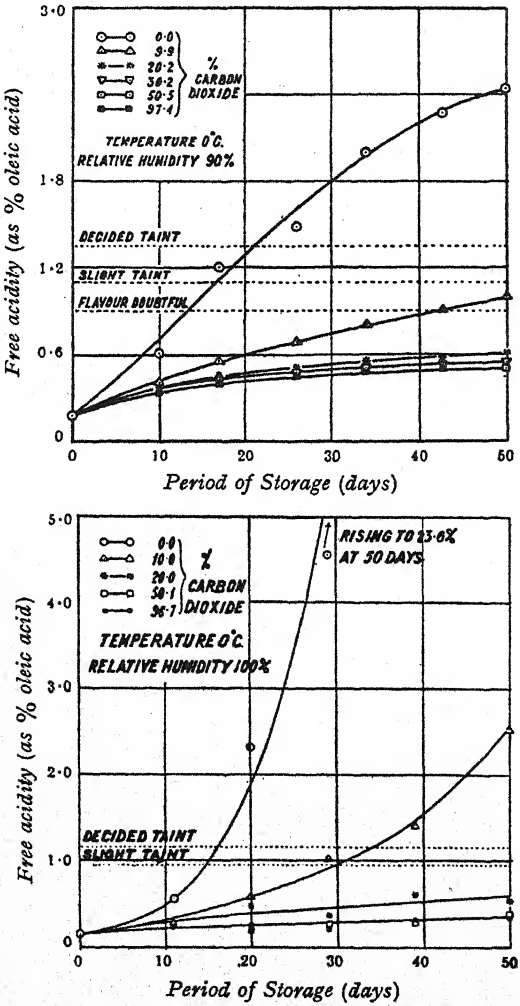


FIG. 10.—*The effect of carbon dioxide on the development of taint in beef-fat at* 0°C. (Lea[45])

rancidity in the fat. Bacon therefore keeps well in 100 per cent. nitrogen or hydrogen, though finally anærobic taint develops more rapidly than in 100 per cent. carbon dioxide. Low concentrations of carbon dioxide reduce microbial growth on bacon, but do not prevent oxidative rancidity.

In many cases the use of carbon dioxide permits storage at higher atmospheric humidities than would otherwise be possible, thus reducing the excessive loss of weight and deterioration in appearance caused by too rapid drying. Storage of chickens in high concentrations of carbon dioxide at 0° C. inhibits microbial spoilage, but in this case the storage-life of the birds is terminated by softening and discoloration, due to autolysis of the abdominal wall by the enzymes of the gut[58, 46]. Gas-storage has been found of value for controlling mould and bacterial spoilage in various other products, including eggs and oil-bearing seeds, and will probably find increasingly varied application in the future.

Storage in Oxygen

It is well known that the majority of micro-organisms, including those for whose growth oxygen is essential, are unable to grow in very high concentrations of this gas. Storage in atmospheres of pure oxygen, particularly under pressure, should therefore increase the storage-life of products susceptible to microbial spoilage. This principle has not yet been applied to the preservation of food except, very recently, in the case of milk and cream. In its present form the Hofius process is useful for the maintenance of relatively small quantities of these commodities in the " fresh " condition—on board ship for example—rather than for large scale commercial practice.

The freshly pasteurised milk or cream is placed in a stout stainless-steel vessel of about 10 gallons capacity, fitted with a pressure-gauge. Oxygen is admitted from a cylinder until the pressure rises to 10 atmospheres, after which the pressure is released and the gas allowed to escape. The vessel is then refilled to the same pressure and the valve closed. Storage is usually at 5° C., the vessel being turned over now and again to minimise separation of the fat. The contents can be drawn off as required. Stored in this way milk or cream remains in good condition from a bacteriological point of view, for five to eight or more weeks, and keeps for the normal length of time when withdrawn from the tank. It is said, however, to possess a slight, though not serious, " oxidised " flavour, due to oxidation of the milk-fat.

The Use of Ozone

Ozone, in common with other powerful oxidising agents, is strongly germicidal towards micro-organisms. It is easily and cheaply prepared by passing dry air between concentric cylindrical metal electrodes a few millimetres apart and at an alternating difference of electrical potential of 5,000–9,000 volts. One of the

electrodes is covered with a glass dielectric in order to eliminate sparks* and to give a uniform, quiet, purple discharge. At a frequency of 50 cycles per minute a yield of about 50 g. of ozone per hour per sq. metre of either electrode is obtained[19].

Ozone is rapidly destroyed in contact with organic matter, and when the supply is shut off the concentration in the chamber falls, being reduced to half in about 30 minutes in a room full of eggs, and in about 15 minutes in a room containing meat. The latter figure refers to meat which has been exposed to the gas for some days, destruction during the first few hours being much more rapid. A suitable concentration of ozone can therefore be maintained by regulating the input of the gas. The concentration at a particular point within the room is determined approximately from the time required for a wetted starch-iodide paper to darken to match a standard.

The use of ozone for the storage of food is limited by the fact that it cannot be breathed in concentrations above about 1 part per million for any length of time without injury to the respiratory tract, and that it is liable adversely to affect the flavour of foods, particularly those of high fat-content.

Exposure to high concentrations of ozone for long periods is necessary to *destroy* moulds. Thus, spores of four species have been found still to be capable of germination after exposure to 400 parts per million of ozone for 10–15 hours, but were killed within 20 hours. On the other hand, much smaller concentrations inhibit germination and arrest growth. Spores sown on to bacon, sausage, etc., failed to germinate at 9° C. in atmospheres containing as little ozone as 0·5 parts per million, and a similar concentration arrested the growth of hyphæ already present. Somewhat higher concentrations were necessary at higher temperatures[48].

The amount of ozone required to produce a particular effect, however, varies very greatly with the experimental conditions. Factors such as the amount of nutrient and organic material present, and the situation of the spores—whether on the surface or protected by a thin film of the medium—profoundly affect the results. For this reason data reported in the literature are very conflicting. Haines[30, 32], working at 20° C. with bacteria on synthetic media containing no derivatives of protein, found that 4 parts per million produced some inhibition and 10 parts per million was germicidal when the gas was introduced simultaneously with inoculation. Much higher concentrations (200–700 parts per million) were required to arrest well established growth, particularly in media containing protein. At 0° C. a concentration of 10 parts per million in the atmosphere caused some inhibition of growth, even in media rich in protein. Bacteria vary considerably in susceptibility to ozone. *Pseudomonas* and *Achromobacter*, which are among the organisms

* Sparks cause oxides of nitrogen to be produced and reduce the yield of ozone.

more susceptible to inhibition by carbon dioxide, are relatively resistant to ozone.

Small amounts of ozone undoubtedly reduce microbial spoilage in some foodstuffs. Organisms present on eggs, for example, have little protection from the gas, and eggs stored at 0° C. in a saturated atmosphere containing 3 parts per million of ozone for three months have been found to show very greatly reduced spoilage by mould as compared with controls stored in air[30]. The eggs in this case had acquired no foreign flavour. On the other hand, eggs stored in 10 parts per million of ozone for five months possessed a definitely unpleasant " metallic " flavour, particularly in the yolk. At 90 per cent. relative humidity, eggs stored in 0, 1 and 5 parts per million of ozone remained free from *external* mould for 54, 90 and >180 days, though *internal* mould was frequently present in the last case. Cooking tests showed that ozone prevented development of the " musty " odour observed in eggs stored in air, and no definite " off " flavour due to the ozone could be detected[49].

On the commercial scale ozone is quite largely used in the storage of eggs, particularly in the United States, a higher humidity in the store, and consequently reduced shrinkage, being rendered possible without loss by mould. In this case the rooms are rarely opened between the entry of the eggs and their removal months later. A continuous concentration of about 1·5 parts per million (by volume) in the aisles, and not less than 0·6 parts per million in the centre of piles is said to ensure protection against mould at humidities up to nearly 90 per cent. This requires for maintenance an input of about 1 g. of ozone per hour for each 30,000 cu. ft. of gross capacity in the store. The eggs are found to be in good condition after eight months, and no foreign flavour has been detected after seven months, even with 3 parts per million of ozone[19].

Small quantities of ozone have been employed in continental slaughter-houses and meat stores for some years. Typical concentrations used are 1·5—3 parts per million during cooling of the carcase (18 hours), and about 1 part per million during subsequent storage. A few meat-rooms in America are said to employ ozone at a concentration of 2–3 parts per million (after the first half hour) for two periods of two hours per 24 hour day. In one store, for example, meat is held for eight weeks at 3° C. and 90–92 per cent. humidity without loss, the amount of ozone required being about 1 g. per hour per 1,500 cu. ft. of space. It is recognised, however, that the formation of bacterial slime on smaller cuts of meat with a fairly high initial contamination cannot be controlled by these methods.* Quantities of ozone of the order quoted above are said not to affect

* Haines [30] finds that continuous exposure to ozone at a concentration of 10 parts per million fails to show any inhibiting effect on the formation of slime on cut surfaces of lean meat at 0° C. and 100 per cent. relative humidity. These conditions, however, are considerably more favourable to bacterial growth than commercial storage of carcases, where extensive superficial drying takes place.

the flavour of the meat, though too high a concentration or exposure for too long a period produces rancidity.

Opinion as to the effect of small amounts of ozone on flavour is by no means unanimous. Lewis and Yesair[48] found that exposure to concentrations as low as 0·3 parts per million of ozone for four days produced "off" flavours in the superficial layers of meat and meat-products, as well as in lard, butter and cheese. Fats in particular acquired a disagreeable "cucumber" odour and increased susceptibility to oxidative rancidity, while a "metallic" taste was produced in all the materials tested. Lea has confirmed the production of these flavours in egg-yolk, egg-white, butter and pure, extracted fats, and has shown that ozone undoubtedly increases the susceptibility of pure fats to oxidation (page 154). These data, however, refer to a concentration of 5 parts per million of the gas; data have not been obtained for lower values. In the practical case the effect produced will be influenced by the area of surface exposed and by the volume and rate of circulation of the air, as well as by the concentration of ozone in use. It is difficult therefore, from the information at present available, to assess the value of ozone for the preservation of food. Obviously care is necessary in its use, particularly with products of high fat-content*.

Ultra-violet Light

Physical methods other than heat can be used for the destruction of undesirable micro-organisms. Bacteria present in food are killed by the application of pressures of the order of 100,000 lbs. per sq. in., or by exposure to sound waves of high frequency or to an electric current. Vegetative organisms and, less easily, spores are destroyed also when the fluid containing them is passed through a tube or round a rod which is electrically vibrated at about 10,000 cycles per second. None of these methods appears to have been used other than experimentally. α, β- and γ-rays from radioactive substances, X-rays and ultra-violet light all possess marked bactericidal properties, but only in the last case has any practical application been attempted.

The effectiveness of ultra-violet light in killing micro-organisms is comparatively low at wavelengths above 3,100 Å, but much greater between 2,900 and 2,250 Å. Radiation below 2,250 Å is probably even more efficient. The following data have been given for *B. coli*[69].

Wavelength (Å) ..	3132	2900	2803	2699	2652	2536
Incident energy required to produce 50% death (ergs per sq. cm. × 10^4)	52	3·4	2·4	1·6	1·1	2·0

The more effective rays are therefore almost absent from sunlight—completely so after the light has passed through glass—but are produced freely by carbon or mercury-vapour arcs. The "cold" type of mercury-in-quartz arc which runs at only a few degrees

* Ozone should *not* be used in chambers containing butter.

above room-temperature emits over 90 per cent. of its radiation at 2536 Å. Radiation of this frequency is highly bactericidal; the emission from a lamp taking (with transformer) 50 watts sufficing to kill half* the bacteria in an aqueous suspension at a distance of 50 cm. in a few seconds.

Various uses have been suggested for lamps of this general type, and a number of experimental installations has been put into operation in the United States[39]. Such uses include the accelerated conditioning or " ripening " of meat at temperatures and humidities not practicable without control of microbial growth, and the " pasteurization " of milk without heat. The surface of cakes during cooling, and the inner surface of wrapping papers as they leave the roll, have also been irradiated in order to kill the spores of moulds.

Two difficulties attend the use of this method in practice. In the first place, the efficiency of the light in killing micro-organisms present on materials such as meat, fruit, etc., is enormously less than for the same organism on a glass plate or suspended in water. This may be due in part to increased resistance of the organisms in presence of the nutrient, but is probably largely a result of the very low penetrative power of radiation of this wavelength. Nevertheless, exposures sufficiently long to reduce greatly the microbial population could be given in many cases, either as one initial " sterilization," as periodical exposures, or by continuous irradiation at low intensity. The second difficulty, however, still remains, namely, the danger that chemical changes due to the light itself, or to the ozone and hydrogen peroxide produced by its action on air and water, might result in " off " flavours in the food. Here, as with ozone, it is necessary to ascertain for a particular set of conditions whether micro-organisms can be usefully reduced without introducing another form of spoilage. As pointed out elsewhere (page 145), ultra-violet light powerfully accelerates the oxidation of fats. The relative influence of various wavelengths within this band has apparently not been determined, but light below about 2,600 Å is powerfully absorbed (Fig. 22), and may quite probably bring about degradation of the saturated hydrocarbon chain in addition to the more normal peroxidic oxidation of the unsaturated constituents.

Certainly beef-fat, both extracted and in the tissue, has been found to develop an unpleasant odour and flavour on exposure to the light of a " cold " mercury arc[44]. The effect produced is unusual, in that a rancid flavour is observed in a sample several millimetres in thickness at very low values of peroxide, owing probably to the formation of an extremely thin superficial zone of highly oxidised fat over a residue of unchanged material. Protein (egg-white) also develops an " off " flavour when exposed to the lamp, but less readily than fat.

Further work is being done with lamps in envelopes of special glass which eliminates the shorter wave-lengths mainly responsible

* The sterilisation-curve is roughly exponential.

for the production of ozone, though still passing fairly freely the main radiation at 2536 Å[44].

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PART V.—THE DETERIORATION OF FATS BY ATMOSPHERIC OXIDATION

SECTION I.—THE OXIDATION OF FATS

The most important, and from a scientific point of view the most interesting form of rancidity is that produced by the action of oxygen of the air on the fat. Decomposition by micro-organisms can only occur when the necessary moisture, nitrogenous substances and mineral salts are present, and within a comparatively narrow range of temperature. Atmospheric oxidation occurs spontaneously when any material containing unsaturated fat is exposed to the air, though naturally the rate of change varies enormously with the type of fat and with the conditions of storage.

The property of undergoing spontaneous oxidation when exposed to the atmosphere is by no means peculiar to edible fats, but is exhibited also by many other substances of biological and industrial importance. Examples are the autoxidation of aldehydes, alcohols, sulphydryl compounds, amino acids, inorganic sulphites, phenols, amines and unsaturated hydrocarbons. Industrially important cases outside the field of the preservation of food are the ageing or perishing of rubber, the formation of " gum " in petrol, the production of sludge in mineral lubricating and transformer oils, and the oxidation and resinification of essential oils. These are all forms of closely related phenomena, arising initially from the action of atmospheric oxygen on the unsaturated centres of the various substances. Oxidative rancidity is of importance in soaps, textile oils, and other inedible fatty products. The formation of gummy exudations on the surface of leather dressed with oils and the drying of paints are also results of atmospheric oxidation.

Effects of Atmospheric Oxidation

Palatability

The symptoms of oxidative rancidity vary considerably with the type of foodstuff, and to some extent with the conditions under which rancidity has developed. A slight " flatness " or " oiliness " of flavour is often the only effect noticeable in the earlier stages, and even this may appear to be due as much to destruction of the natural fresh aroma and taste, as to development of a true foreign flavour. Later, odour and flavour become definitely objectionable, and the fat is usually described as " tallowy." When rancidity has reached a sufficiently advanced stage the odour frequently takes on a pungent, acrid quality, reminiscent of the oxidised linseed-oil of drying paint. General rules, however, cannot be given for the effect of the oxidation of fat on the flavour of the food containing it. It is sufficient that the result, whether described as a " cardboard " or an " oily " flavour in milk, a " fishiness " or " tallowiness " in butter, cream, ice-cream, or dried milk, a " bitter " taste in cereal products,

or a " rancid " flavour in nuts, potato-chips, or bacon, is invariably unpleasant and often sufficiently so to render the foodstuff inedible.

Colour

Loss in palatability, though the most obvious and perhaps the most serious, is by no means the sole result of this form of rancidity. The reactive peroxides produced during oxidation are much more potent as oxidising agents than atmospheric oxygen itself, and are capable of bringing about or accentuating other undesirable changes in non-fatty constituents of the food.

Fats and oils, as they occur in the tissues, are usually accompanied by pigments, often carotinoid in nature, which are destroyed as oxidation proceeds. When the fat possesses a well-defined induction-period, bleaching tends to occur fairly sharply at the conclusion of this stage, as the second phase of rapid oxidation sets in (Fig. 14).* Oxidative destruction of the pigments of crude vegetable oils by air, sometimes in the presence of light, is the basis of several commercial processes of bleaching[296]. A similar result can be achieved more rapidly by the addition of small quantities of benzoyl peroxide to the heated fat[145].

Carotene is much more stable towards oxidation when dissolved in natural oils than when in solution in unoxidisable solvents such as the methyl esters of saturated fatty acids[209]. The unoxidised natural oils, or more probably the antioxidants which they contain, are apparently able to protect carotinoids, while oxidised oils, which contain peroxide and no antioxidant, rapidly destroy them.

Vitamin-content

The growth-promoting, anti-ophthalmic vitamin A present in oils and fats is known to be readily destroyed by atmospheric oxidation[96], and vitamin E, which is essential for normal fertility and reproduction in animals, also disappears as the oil containing it becomes rancid[205, 55]. Vitamin E *concentrates*, however, have been found to retain their activity for some weeks in food-mixtures containing oxidised fat. Vitamin D is normally resistant to oxidation, but in sufficiently extreme cases some loss even of this vitamin has been found to occur (Table 26).

Cod-liver oil is now used on quite a large scale, both as a means of increasing the vitamin-content of human diets, and in the raising of poultry and stock as a supplement to the winter feed. In both cases its purpose is to make good deficiencies of vitamins A and D caused by lack of fresh green food and sunlight. Medicinal oils are frequently allowed to become rancid through improper storage before being finally consumed, whereby an appreciable proportion of their vitamin A potency is lost. As can be seen from Fig. 11, vitamin A-activity falls and peroxide-content rises as the oil oxidises, the actual

* A transient very large *increase* in depth of colour has recently been observed in certain vegetable oils, oxidising at 100° C., immediately prior to bleaching [311, 95]. No explanation has yet been given for this behaviour.

relationship between these values varying with temperature, access of oxygen and exposure to light. A recent publication[203] demonstrates a relationship between destruction of vitamin A and end of the induction-period (peroxide-value) in halibut-liver oil precisely

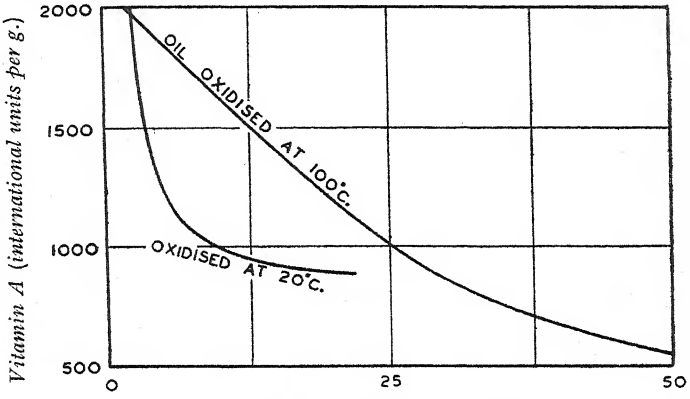


FIG. 11.—*Oxidative destruction of vitamin A in cod-liver oil at* 20° *and at* 100° *C.* (Whipple[313])

similar to that already observed for the bleaching of carotene in butter-fat (Fig. 14).* Low-grade, oxidised cod-liver oils are unsuitable for feeding to stock, and even an oil of good quality will rapidly lose its activity if incorporated in finely divided feeds for some time

TABLE 26.—*Effect of storage on the vitamin D-activity of cod-liver oil.* (Norris, Heuser and Wilgus[226])

Feed.	Rachitic lameness (%) in chicks at age (weeks)					
	3	4	5	6	7	8
Fresh oil	0	0	0	0	0	0
Stored 4 weeks ..	0	0	0	0	10	16
Stored 8 weeks ..	0	8	8	16	24	28
Stored 12 weeks ..	5	19	32	40	39	43
No oil	3	44	81	100	100	100

before use. Table 26 gives data from an experiment in which the efficiency of cod-liver oil, fed as a source of vitamin D to chickens receiving an otherwise adequate diet, decreased with increasing time of storage of the feed containing it.

* Fish oils apparently contain their vitamins partly free and partly in combination as esters of fatty acids. Free vitamin A is rapidly destroyed on aeration, but its esters are relatively stable. (Hickman, K.C.D. *Industr. Engng. Chem.*, 1937, **29**, 1107.)

Some of the anomalous results obtained in the earlier days of research on vitamins, particularly when minerals containing iron had also been added to the diet, were undoubtedly due to effects of this kind.

Toxicity

Evidence has recently been obtained that oxidised fats, in addition to being inferior to the fresh material in palatability and nutritive value, may actually be injurious to health, and lead to digestive[315] and other disturbances. Whipple[312] has found that dogs, fed on a diet containing oxidised fat, developed a disease which she termed the "oxidised fat syndrome," and ultimately died, while control animals, receiving a similar ration containing unoxidised fat, remained in good health. The material used was lard through which oxygen had been bubbled at 60° C. until it gave a decided colour in the Kreis test, but none when diluted 10 times with purified paraffin. The experiment has since been repeated on rats, with similar results[313]. The lard in this case was oxidised with air at 70° C. to a peroxide-content of 15–20 ml. 0·002 N. thiosulphate per gram. Kreis and peroxide values of this magnitude correspond to only slightly rancid fats of hardly perceptible odour. These findings have not yet been confirmed for other animals or for man, but they certainly suggest that some physiological justification exists for the dislike which most people have for rancid fats.

Highly oxidised fats have been found to be more effective as fungicides than either hydrogen peroxide or sodium hypochlorite[126]. In this respect they appear to behave similarly to peroxidised essential oils, which are already employed to some extent as germicides.

The Action of Oxidising Agents

Before proceeding to a description of the complicated series of changes which occurs when a fat becomes rancid through oxidation, it will be advisable to consider briefly the effect of chemical oxidising agents on the simpler fatty acids.

Hydrogen Peroxide

The majority of weak oxidising agents have no effect on the saturated fatty acids, while more vigorous treatment breaks them down, mainly to carbon dioxide and water. Dakin[57], however, by heating the ammonium salts of a number of the higher saturated fatty acids for some hours with aqueous hydrogen peroxide, obtained small yields of the methyl ketone containing one carbon atom less. This observation linked up excellently with the theory of β-oxidation deduced from Knoop's feeding experiments, according to which fatty acids break down in the body by a series of stages each involving the loss of two carbon atoms.

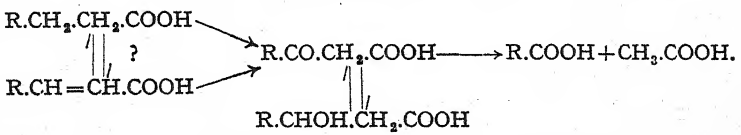
More recently ketones have been isolated in the hydrogen-peroxide reaction, which proves that γ- and δ- as well as β-oxidation

occur[44]. Smedley-Maclean and Pearce[271] have also repeated Dakin's experiments, but with the addition of a copper salt as catalyst. Under these conditions evidence was obtained of hydroxylation in the β-, γ- and δ-positions, and unsaturated hydroxy acids and volatile acids of lower molecular weight were formed in considerable yield. Ketones, however, were now present only in traces.

Modern work on the metabolism of fat indicates that β-oxidation is not the only form which occurs *in vivo*; evidence has been obtained that the fatty-acid chain can be attacked in the α-, γ-, δ- and even ω- positions, with production in the last case of dicarboxylic acids. According to the "multiple alternate oxidation theory" recently proposed by Jowett and Quastel,* the fatty acid, absorbed at an enzymic surface, is considered to undergo oxidation at alternate carbon atoms throughout the chain before the latter breaks down to acetoacetic acid and other acid products.

$$CH_3.(CH_2)_6.COOH \longrightarrow CH_3.CO.CH_2.CO.CH_2.CO.CH_2.COOH \longrightarrow \text{etc.}$$

The following scheme illustrates the structural relationships between some of the products of oxidation, but the precise route by which each is produced from the parent acid is not certain.

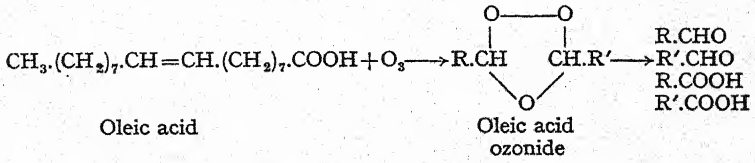


The similarity between the products of oxidation by hydrogen peroxide and by certain moulds has already been pointed out (page 57).

Concentrated hydrogen peroxide (perhydrol) in a neutral solvent such as acetone, reacts slowly with oleic acid with production of small quantities of the 9 : 10 dihydroxystearic acid melting at 95° C. The action of hydrogen peroxide in acetic acid solution (peracetic acid) is described below.

Ozone

Ozone reacts with unsaturated fatty acids, adding on at the double bonds to produce ozonides[127]. These are oily, unstable substances which readily break down on treatment with water or dilute acids to yield aldehydic and acidic decomposition products.



* *Biochem. J.*, 1935, **29**, 2143, 2159.

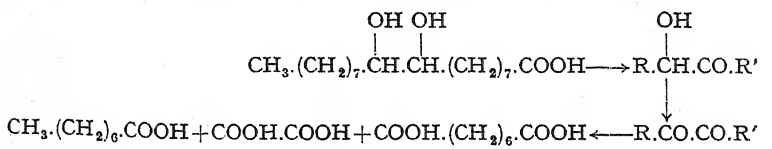
Potassium Permanganate

The products of the oxidation of unsaturated fatty acids with potassium permanganate vary according to the experimental conditions.

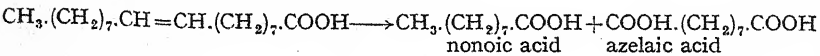
(*a*) Oleic acid in ice-cold alkaline solution is rapidly oxidised by the calculated quantity of dilute permanganate to dihydroxystearic acid.

The dihydroxy acid (m.p. 132° C.) is obtained in excellent yield from oleic acid. Linoleic (two double bonds) and linolenic (three double bonds) acids give progressively smaller yields of the tetra- and hexahydroxy acids (40 per cent. and 15–18 per cent. respectively[103]).

(*b*) Carefully controlled oxidation of the dihydroxy acids yields ketohydroxystearic and diketostearic acids[138], and finally, with rupture of the chain, caprylic, oxalic and suberic acids[178]



(*c*) Powdered potassium permanganate in boiling acetone solution breaks the carbon chain at the double bond, with production of acidic decomposition products[11,133]. In this reaction a certain amount of intermediate product in which the double bond has been saturated but not broken, is always obtained, necessitating a second treatment with permanganate.



Peracetic and Perbenzoic Acids

Hydrogen peroxide in glacial acetic acid oxidises unsaturated fatty acids, the first stage of the oxidation being, presumably, the addition of peracetic acid at the double bond, followed by a certain amount of hydrolysis to the dihydroxy acid[134]. The hydrolysis is readily completed by treatment with dilute alkai. In addition to these compounds, a considerable proportion of ill-defined, oily product of oxidation is always obtained.

Perbenzoic acid reacts with unsaturated fatty acids in a somewhat different manner, the first product being usually a crystalline oxido acid which can be converted quantitatively into the dihydroxy compound by hydrolysis with dilute acid[25].

$$CH_3.(CH_2)_7.CH{=}CH.(CH_2)_7.COOMe \longrightarrow R.\underset{\diagdown\,O\,\diagup}{CH-CH}.R' \longrightarrow R.\overset{OH}{CH}-\overset{OH}{CH}.R'$$

The hydroxy acid obtained in both cases from oleic acid melts at 95° C., and is probably the *cis* geometric isomer. That resulting from the action of permanganate (m.p. 132° C.) corresponds to the *trans* acid (elaidic), inversion having occurred during the oxidation.

The Action of Oxygen on Fats

An oil or fat consists of a complex mixture of glycerides, which are in turn built up from glycerol in combination with saturated and unsaturated fatty acids. Under ordinary conditions saturated fatty acids are stable in air. Myristic acid, for example, does not autoxidise on heating for 25 hours in oxygen at 90° C.[299] Palmitic and stearic acids have even been recovered from Egyptian rock-tombs, still in much the same proportion as when first incorporated, probably as beef-tallow, in cosmetics buried 3,000–5,000 years ago[16]. Most of the unsaturated acids originally present had broken down during the course of ages, and the fragments in their turn had largely volatilised. It is probable, however, that under the influence of the peroxide present in oxidising fats, a certain amount of oxidation

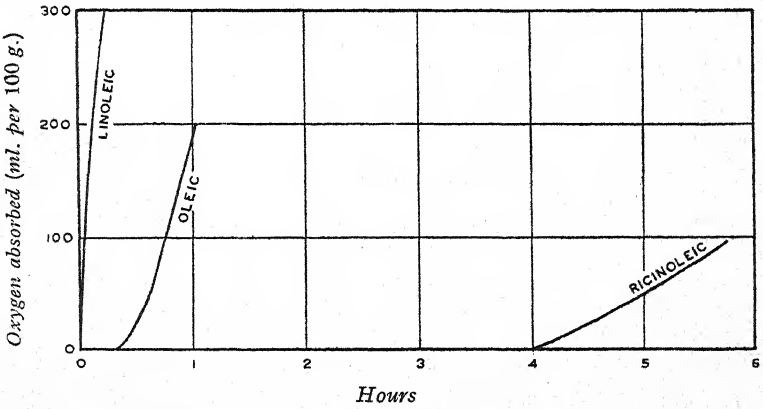


FIG. 12.—*Relative rates of oxidation of unsaturated fatty acids at* 95° *C.* (Holm, Greenbank and Deysher[142]).

of saturated acids *does* occur. Free oxygen, in the presence of short-wave ultra-violet light or of metallic catalysts at elevated temperatures, is known to oxidise saturated acids directly.

Under normal conditions the amount of chemical change necessary to make an edible fat rancid is very small. A perceptible "off" flavour can be observed in beef-kidney or butter fat when

only about one-thousandth part of its substance has undergone chemical change. In more highly unsaturated fats the amount of change necessary may be much greater, but is still small.

Fatty acids in general tend to become more reactive towards oxygen as the number of double bonds in the molecule increases. Linoleic acid, for example, oxidises much more rapidly than oleic acid under the same conditions[293, 142] (Fig. 12). In the presence of haemin as catalyst the relative rates of oxidation of oleic, linoleic and linolenic acids have been found to be of the order of 1, 12, and 100[174]. The enormous variation in stability towards oxidation shown by natural oils and fats from different sources (Fig. 19) is thus partly (but not entirely) accounted for by differences in the proportion and degree of unsaturation of the constituent fatty acids.

Susceptibility to oxidation is also influenced to some extent by molecular structure. Elaidic acid, for example, is much more resistant (50 times according to Kuhn and Meyer) than its geometric isomer oleic[294, 77], while introduction of an OH group into the oleic acid chain to form ricinoleic acid, $CH_3.(CH_2)_5.CHOH.CH_2.CH=CH.(CH_2)_7.COOH$, results in a great decrease in susceptibility (Fig. 12). The relative stability of ricinoleic glycerides towards oxidation has been an important factor in establishing the value of castor-oil as a lubricant.

The influence of position in the chain on the rate of atmospheric oxidation of a double bond has not yet been studied in detail, but on theoretical grounds it is probable that reactivity towards oxygen will vary in some degree with proximity to other unsaturated centres, and to the carboxyl group. In the case of monomolecular films of three of the isomeric monoethylenic acids, oleic ($\Delta^{9:10}$), petroselinic ($\Delta^{6:7}$), and iso-oleic ($\Delta^{2:3}$), oxidation by permanganate has been found to proceed much more rapidly in the last than in either of the other two cases. Chaulmoogric acid ($C_{18}H_{32}O_2$), in which one double bond is part of a five-membered ring at the end of the chain remote from the carboxyl group, also oxidised very rapidly[149]. On the other hand, monoethylenic acids in which the double bond is in the chain at the end remote from the carboxyl group, have been found to be completely resistant to the action of oxygen in the presence of haemin as catalyst[174].

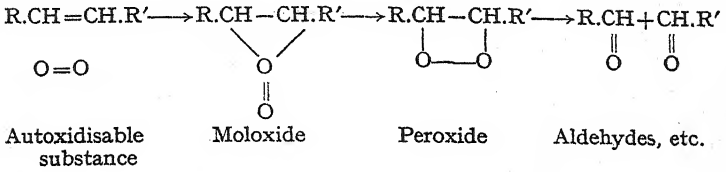
Mechanism of the Oxidation

Autoxidation of an organic substance with a carbon to carbon double bond was recognised by Schönbein in the case of turpentine as early as 1858, and modern theories as to the mechanism of reactions of this type date from the work of Bach[12] and of Engler and Wild[83] in 1897.

Formation of Peroxide.—Many organic compounds, including the unsaturated fatty acids, oxidise spontaneously when exposed to the air, oxygen attacking a double bond with the formation of a highly

reactive peroxide. This primary addition product is almost invariably unstable, and decomposes readily by polymerization or isomerization, or by splitting somewhat after the manner of an ozonide to produce compounds of lower molecular weight. Only in comparatively few cases, such as those of ergosterol, rubrene and tetrahydronaphthalene, has the peroxide been isolated in the pure state. That from tetrahydronaphthalene, for example, is a crystalline substance which has been shown to possess a structure corresponding to the addition of a molecule of oxygen to one of the double bonds[136].

Staudinger[276] believes that peroxides of this type are *not* the first products of the reaction, but that they are preceded by extremely unstable and highly reactive substances to which he assigns the term "moloxide" and the possible formula given below.*



Oxidised fatty acids and glycerides give many of the reactions characteristic of peroxides, but the fatty peroxides themselves are non-crystallisable, unstable substances which have not been isolated.

TABLE 27.—*Formation of peroxide by addition of two atoms of oxygen per double bond.*

(Goldschmidt and Freudenberg[102])

Substance.	Oxidised by.	Original ester.	Oxidised ester.		
		Iodine-value.	Iodine-value.	Per-oxide.	Total.
Methyl linolenate	Shaking in air	259	163	89	252
Linolenic acid ..	,, ,,	274	135	150	285
Linseed oil ..	,, ,,	180	131	22	153
,, ,, ..	,, ,,	180	132	24	156
,, ,, ..	,, ,,	180	132	22	154
Methyl linolenate	Standing in air	259	220	40	260
Linolenic acid ..	,, ,,	274	121	148	269

* Staudinger's moloxides bear some resemblance to the "activated" peroxide molecules of the modern chain theory (page 137).

Various observations[76, 202] indicate that drying oils take up at least two atoms of oxygen per double bond, but in the majority of the experiments a considerable amount of secondary decomposition and further oxidation must certainly have occurred. Oleic acid at 80°C. absorbed approximately *four* atoms of oxygen per molecule, with a low yield of peroxide and a considerable evolution of water and carbon dioxide[124].

On the other hand, in the case of linolenic acid or its methyl ester oxidising at room temperature in the presence of a cobalt catalyst, absorption of oxygen, disappearance of the double bonds, and accumulation of peroxide, all corresponded to the addition of two atoms of oxygen to each double bond (Table 27). Trilinolenin took up nearly 18 atoms of oxygen without appreciable formation of secondary products. Linseed-oil, however, in common with other fats, always showed considerable decomposition of the peroxide.

Decomposition of the Peroxide.—The precise manner in which the peroxide decomposes is still largely a matter for conjecture, and several schemes differing in detail have been proposed to account for the numerous products isolated.

Powick[236], applying the theories of Engler and of Bach, suggested that the peroxide might be capable of oxidising the fatty-acid chain with removal of two hydrogen atoms to form a new double bond, being itself thereby reduced to an oxide. The formation of peroxide, followed by splitting at the new double bonds, could then account for the series of saturated aldehydes and acids (most of the members from C_1 to C_9) which have been found in oxidised fats[260], as well as for epihydrin aldehyde, the substance responsible for the Kreis test (page 99).

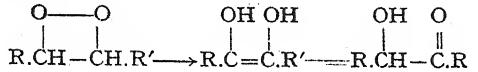
The theory proposed by Tschirch[301] differs from that of Powick in that the peroxide is considered to be decomposed by water with production of an oxide, hydrogen peroxide* and ozone, the ozone then reacting with other unsaturated acid molecules to form ozonides which, in the presence of water, decompose into aldehydes and acids.

Browne[34] suggested a mechanism according to which a molecule of oxygen reacts with a double bond to produce a fatty oxide, with simultaneous liberation of an atom of active oxygen. This attacks the glycerides (1) to form a further molecule of oxide, (2) by dehydrogenation of the chain, with production of secondary double bonds, or (3) *via* intermediate formation of hydrogen peroxide, to produce hydroxy and ketonic compounds which subsequently decompose with rupture of the chain. The oxide is considered to isomerise to a ketone, which then breaks down on hydrolysis into substances of lower molecular weight.

* Hydrogen peroxide has been shown to be present in oxidising fats [159].

Oxido- and dihydroxy-stearic acids do not readily react with molecular oxygen[124], but would perhaps be further attacked by the reactive peroxides of an oxidising fat. Crystalline dihydroxystearic acid has been isolated from rancid lard[259] and from rancid oleic acid[270], and oxidoelaidic acid from the autoxidation of oleic and elaidic acids in the presence of a cobalt catalyst[78]. In the latter case azelaic, suberic, oxalic, nonoic and caprylic acids were also identified, indicating that a considerable amount of oxidative decomposition, analogous to that produced by the action of potassium permanganate (page 84), had occurred.

Ellis[76] postulated an intramolecular rearrangement of the peroxide to a tautomeric ketohydroxy-dihydroxy system, and produced experimental evidence in support of this view by identification of considerable quantities of hydroxyl and keto groups in films of dried linseed oil.

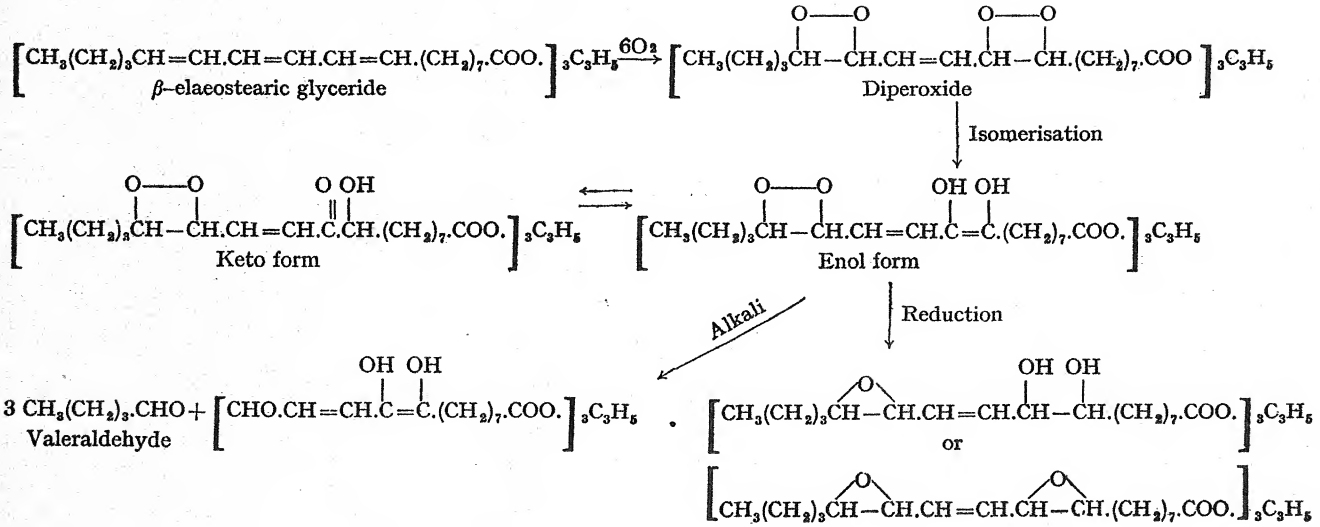


Though differing in detail, all of these theories agree in requiring the primary formation of an oxide or peroxide, which then either breaks down directly into aldehydes and acids of lower molecular weight, or isomerises, or reacts with water to form substances containing hydroxyl and keto groups. Proof of rupture at the double bond is provided by the isolation of azelaic and nonoic acids from rancid cottonseed-oil[225], lard[260] and oleic acid[270]

The Oxidation of β-Elaeostearin.—The theories of the oxidation of fat have received striking confirmation from the experimental work of Morrell and Marks[215] on β-elaeostearin, a pure crystalline glyceride, prepared from Chinese wood (tung) oil. From the product obtained by complete oxidation of this glyceride, they succeeded in isolating a substance which they showed to contain one peroxide, one carbonyl and one hydroxyl group, in addition to one unoxidised double bond, per molecule of acid.*

This substance, on treatment with methyl alcohol and gaseous hydrochloric acid, gave rise to two series of methyl esters, derived respectively from the keto and enol forms of the peroxide adjacent to the carboxyl group. On reduction with hydrogen and palladium the peroxide remote from the carboxyl group was apparently converted into a glycide (monoxide) grouping, the double bond of the enol form of the near peroxide being at the same time saturated. Treatment with hydrogen iodide reduced both peroxide groups to monoxide. The diagrammatic scheme on page 90 summarises the changes involved.

* The middle component of the system of three conjugated double bonds in the elaeostearic acid molecule is relatively inert towards oxygen.

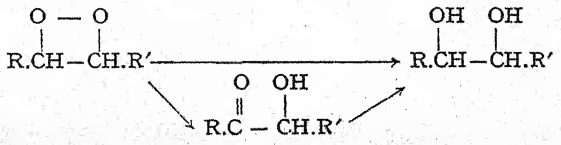

β-elaeostearic glyceride
6O2
Diperoxide
Isomerisation
Keto form
Enol form
Alkali
Reduction
3 CH3(CH2)3.CHO + [CHO.CH=CH.C=C.(CH2)7.COO.]3C3H5
Valeraldehyde
or

A number of interesting points concerning the behaviour of fatty-acid peroxides arise from this work. In the first place, the two peroxide groups formed by atmospheric oxidation of β-elaeostearin differ very markedly in chemical properties. That remote from the carboxyl group appears to be basic in character, is stable in the presence of acids, and can be distilled *in vacuo* (as ester) without decomposition. It is, however, unstable towards alkalies, which cause rupture of the chain with splitting off of valeraldehyde. The peroxide nearer to the carboxyl group possesses feebly acidic properties and displays keto-enol tautomerism, derivatives of both forms having been isolated.

Further data have since been obtained from the maleic anhydride compounds of both α- and β-elaeostearic acids[2][14]. These substances possess the formulæ · $CH_3(CH_2)_3R.CH{=}CH.(CH_2)_7.COOH$ and $CH_3(CH_2)_3CH{=}CH.R.(CH_2)_7.COOH$ respectively, where R represents a double ring system containing the maleic anhydride residue and the remaining (inert) double bond. The double bonds shown in the formulæ correspond respectively to the "near" and "remote" positions referred to in the previous paragraph. On oxidation the α- (near) compound gave a product which showed no peroxidic properties and appeared to be wholly in the keto- (–CO.CHOH–) form. The β- (remote) compound on the other hand, gave a typical peroxide which liberated iodine from potassium iodide and promoted polymerization and consequent gel-formation.

Gaseous Products of Decomposition.—During the oxidation of unsaturated fatty acids, particularly at high temperatures and in the presence of metallic catalysts, a certain amount of complete breakdown to carbon dioxide and water occurs. Carbon monoxide, volatile acids, and acrolein and other volatile aldehydes, have been detected in the vapours evolved from drying oils. Hydrogen occurs in appreciable quantity among the gaseous products of decomposition obtained by heating peroxidised fat in a sealed tube[6]. The presence of this gas is difficult to account for unless direct desaturation of the fatty-acid chain occurs, possibly as a preliminary to polymerization.

Labile Peroxides.—Banks and Hilditch[15] submitted the product obtained by oxidation of methyl oleate at 100° C. (iodine-value, 16) to reduction with zinc dust and acetic acid, and obtained a yield of approximately 15 per cent. of dihydroxystearic acid in which both the *cis* (m.p. 95°C.) and the *trans* (m.p. 132°C.) isomers were present.



An increase in iodine-value of nine units which occurred during the reduction, was attributed to removal of loosely bound oxygen, with regeneration of the ethylenic ester. Similar increases in iodine-value have been observed on heating *in vacuo* the products obtained by oxidising methyl oleate with peracetic acid[134], or with oxygen at 100°C. [135]

Iodine-values of oxidised oils are sometimes corrected on the assumption that the peroxides present liberate iodine quantitatively during the determination[6]. If this assumption were correct, it might be possible to account for part or all of the observed rise in iodine-value by decomposition of peroxide in the usual way (page 107) without regeneration of the double bonds. Recent work, however, indicates that the presence of peroxide does *not* interfere with estimation of double bonds by the iodine-value method[320, 124]. The experimental data referred to above must therefore mean that some of the oxygen present in an oxidised fat is so loosely bound that it can be removed with regeneration of the double bond when the fat is heated *in vacuo* or subjected to reducing conditions.

Oxidation in Monomolecular Films.—Gee and Rideal[97] have studied the oxidation and polymerization of monolayers of drying oils spread on the surface of aqueous solutions. These authors made use of the maleic anhydride compound of β-elaeostearic glyceride, a substance in which two of the three conjugated double bonds in each acid residue have been blocked by condensation with maleic anhydride.

The remaining double bond (that remote from the carboxyl group) oxidises readily, the labile peroxide subsequently either (a) polymerizing or (b) isomerising, to a more stable form, which polymerizes much more slowly. Since polymerization is favoured by increasing the pressure on the film, while isomerisation is but little affected, it has been possible, by choice of suitable experimental conditions, to investigate the two reactions almost independently of one another. Further application of this method should lead to useful results.

SECTION 2.—THE DETECTION AND ESTIMATION OF OXIDATIVE RANCIDITY.

The majority of the natural oils and fats oxidise with a more or less well defined induction-period, during which absorption of oxygen and change in palatability either cannot be detected or are relatively small. Frequently this phase of the reaction can be divided into two parts. During the first, which may be prolonged, oxidation proceeds at a slow and almost constant rate. This is followed by a second stage during which the velocity of the reaction increases in a logarithmic manner until it attains its maximal value. In some cases, depending on the sample and on external conditions, only this second stage can be observed, and sometimes even this is absent.

The duration of the induction-period as a whole is of the greatest practical importance, since it is upon this that storage-life (from the point of view of oxidation) largely depends. Once the phase of rapid oxidation has set in, a pronounced rancid odour and flavour quickly appear and it is almost impossible to save the fat from spoilage.

Odour and Flavour

The simplest and most obvious tests for deterioration are based on taste and smell, and these are still widely used. They are, however, open to several objections (page 35), not the least important of which is the difficulty of obtaining by this means any numerical expression for degree of rancidity. Detection of the earlier stages of spoilage may be difficult or even impossible in case of fats which have themselves a pronounced natural odour, or are constituents of strongly flavoured food. For these reasons much research has been directed towards the provision of chemical means for the detection and evaluation of oxidative rancidity. Such tests will now be considered. The important group of methods which aim at determination, on the fresh material, of its comparative liability to future deterioration will be dealt with later (page 124).

The mechanism of the reaction already discussed indicates that it should be possible to follow the progress of oxidative decomposition in a fat, either by direct measurement of the quantity of oxygen taken up, or by estimation of one or other of the considerable number of products of the reaction. In fact, it is found that most of the ordinary analytical characteristics of a fat change more or less progressively as oxidation proceeds. Thus, the iodine-value falls, and the refractive index increases, as double bonds are destroyed by oxidation. The saponification-equivalent (mean equivalent weight of all the acids present), acid value (proportion of uncombined acids), and Reichert-Meissl and similar values (proportion of steam-volatile acids), all rise as decomposition of the fat results in the production of acids of lower molecular weight. The acetyl value (proportion of hydroxyl groups) gradually increases with the formation of hydroxy compounds. Other characteristics to show change are specific gravity and viscosity, which increase, and heat of combustion, which falls. A few of these methods have found limited use as criteria of oxidation in investigations in the laboratory (Fig. 13).

Acid Value

Much confusion existed in the earlier literature concerning the connection between the free-acid content of a fat and rancidity. Ritsert (1890), Spaeth (1896) and other early investigators used increase in free acidity as a general indication of the development of "rancidity" Later it came to be recognised that atmospheric oxidation is considerably less important as a potential producer of free acids than moisture and enzymic or bacterial action. Thus,

under certain conditions, e.g., in the earlier stages of a light-accelerated oxidation at room temperature, very little change in free acidity occurs, with the result that a fat may be extremely rancid and yet possess a low acidity[191]. Even at 100° C. the production of free acid does not occur in any appreciable quantity until some time after rapid absorption of oxygen has commenced. (Fig. 13.)

A sudden decrease (0·2–0·4 per cent.) in the small amount of free acid present in fresh fats has been reported to coincide with the appearance of rancidity, being followed by the usual increase[164], but this observation has not been confirmed. The implication that

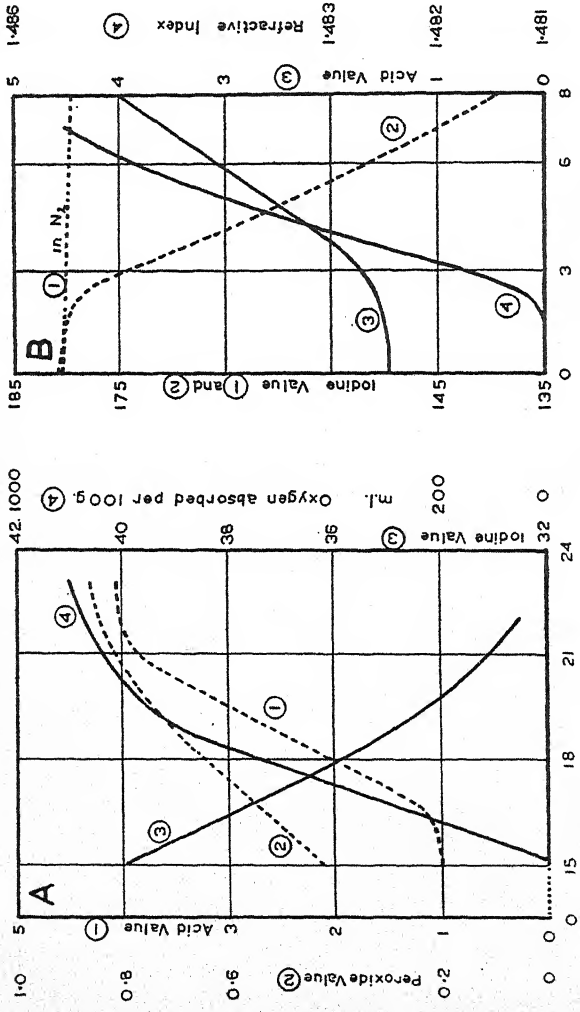


FIG. 13.—*Changes in analytical characteristics during the oxidation of fats. (A) Butter-fat stirred in oxygen.* (Briggs[31]). *(B) Linseed-oil irradiated with ultra-violet light in oxygen.* (Horio[147]).

free acids are more readily oxidised than glycerides is supported by the work of Holm, Greenbank and Deysher[142], who stated that the addition of quantities of free acid, barely perceptible by titration, materially increases susceptibility. Greenbank[105] has recently reaffirmed that saturated acids slightly, and oleic acid greatly accelerate the oxidation of butter-fat. Lea, on the other hand, could find no marked change in susceptibility as a result of increasing the free-acid content of mutton-fat from 0·24 to 0·52 per cent., though very small quantities of peroxide oxygen materially reduced the induction-period[179].

It is true that, in general, a mixture of fatty acids oxidises more readily than the natural oil from which it has been obtained, but it is by no means certain that this cannot be accounted for by loss of antioxidant and by slight oxidation during preparation. Hilditch and Sleightholme[135] found that the neutral glycerides synthesised from the distilled acids of several vegetable oils actually possessed *shorter* induction-periods than the free acids themselves. The statement, almost universal in the literature, that traces of free acid accelerate the oxidation of neutral oils and fats, seems therefore open to question.

Iodine-value

Measurement of the fall in iodine-value has been used extensively in researches on the pro- and anti-oxidant effect of substances added to oils[307, 287], in following the photochemical oxidation of linseed-oil and of linoleic acid[147], and in numerous other instances recorded in the literature. Obviously determination of the iodine-value gives a useful measure of gross chemical decomposition, but it has been shown repeatedly that a relatively advanced state of deterioration, from the point of view of odour and flavour, must be reached before any appreciable change in iodine-value is detectable. This is particularly true in case of fats of the butter and beef fat type, in which oleic is the chief unsaturated acid.

Peroxide-value

A modification of the Fahrion technique for the estimation of peroxides has been successfully employed by Marks and Morrell[215] in examining products from the complete oxidation of β-elaeostearin, but is not sufficiently sensitive for measurement of the traces of peroxides present in rancid edible fats[31]. Substances examined by these authors usually possessed peroxide-contents of the order of 3,000 ml. 0·002 N. thiosulphate per gram, the blank titration being of the order of 220 ml. per gram.

A considerable number of special tests for rancidity have been devised, all of which depend on the chemical detection or estimation of one or other of the substances produced during oxidative spoilage. Those which have found most extensive use are described below, but discussion of the relationship which they bear to rancidity as measured by odour and flavour, is reserved for a later section.

Determination of Acids insoluble in Petroleum Ether

Fresh fats, with very few exceptions, contain practically no hydroxy or oxy-acids, but these appear as a result of atmospheric oxidation. Being non-volatile and relatively resistant to further change, they tend to accumulate as oxidation proceeds. Fahrion[86] and subsequently others[20, 119] have made use of the low solubility of these oxidised acids in petroleum ether as a means of estimating them, and hence the degree of rancidity of fats and soaps. Fahrion's method is carried out as follows :—

3 g. of the fat is saponified with alcoholic potassium hydroxide and the alcohol distilled off. The residual soap is dissolved in 50–70 ml. of hot water and transferred to a separating funnel. When cooled, 100 ml. of petroleum ether are added. The solution is acidified with hydrochloric acid, shaken, and allowed to stand overnight. After running off the aqueous layer, the petroleum ether solution is filtered, the precipitate of oxidised acids washed several times with more petroleum ether and dissolved in the smallest possible amount of warm alcohol. The solution is transferred to a tared dish, the alcohol driven off, and the oxidised acids dried at a temperature which is not allowed to exceed 95° C., and weighed.

Figures obtained for fresh fats ranged from 0–0·17 per cent.; a sample of lard, rancid but not badly so, contained 0·55 per cent. of oxidised acids; a distinctly rancid linseed-oil, 0·87 per cent.; a very rancid lard, 5·25 per cent.; and an extremely rancid sample of soya-bean oil, 27·95 per cent. Little further work appears to have been done on the relationship between content of acids insoluble in petroleum ether and odour and flavour. The method gives reproducible results but is somewhat laborious, and it is doubtful whether its sensitivity is of a sufficiently high order for estimation of the relatively slight changes normally encountered in the fats of food. It is, however, useful for the examination of soaps and of other inedible fatty materials.

Oxidisability-value

Issoglio's method[156], which consists in determination of the quantity of readily oxidisable steam-volatile substances present in a known weight of fat, has found some considerable practical application. Kerr[163], modified the test by using extraction with boiling water in place of steam-distillation as a means of separating the oxidisable substances, and his method appears largely to have displaced the original, though Issoglio[157] considers it in some cases less reliable. The test is applied as follows :—

25 g. of the fat are weighed into a 200 ml. Erlenmeyer flask and 100 ml. of distilled water added. The flask is allowed to stand on the steambath for two hours with occasional shaking, after which the contents are filtered through a wetted filter paper into a 100 ml. graduated flask, cooled, made up to the mark, and mixed thoroughly. To 10 ml. of this solution are added 50 ml. of water, 10 ml. of 20 per cent. sulphuric acid, and 50 ml. of 0·01 N. potassium permanganate, and the mixture is boiled for five minutes under a ground-in reflux condenser. The liquid is then cooled, 50 ml. of 0·01 N. oxalic acid run in, and the unoxidised excess titrated with 0·01 N. permanganate. If T and t represent the volumes of permanganate solution used up in the

oxidation and in a blank test respectively, and W be the weight of fat taken, then the "oxidisability value," which represents the number of milligrams of oxygen required to oxidise the water-soluble constituents from 100 g. fat, is given by $\frac{(T-t)80}{W}$

Lampitt and Sylvester[176] in a recent publication recommend that when the Issoglio value is higher than 40, the quantity of fat used for the determination should be reduced by half. Stirring in order to ensure efficient extraction, and the use of an inert atmosphere to minimise further oxidation during the determination, are obviously improvements on Kerr's method, though stirring by means of a stream of nitrogen appears to result in some slight loss of volatile oxidisable products. The procedure as described by these authors is as follows.

12·5 g. of fat and 50 ml. of boiled and cooled distilled water are placed in a 175 ml. flask provided with a single-surface condenser having a ground-glass joint. A slow stream of nitrogen is bubbled through the contents of the flask by means of a tube passing down the inside of the condenser, and the flask is heated on a boiling water-bath for 2 hours in a location protected from light. Subsequent procedure is as above. It is permissible to make determinations on less than 12·5 g. of fat, provided that the weight taken is increased to 12·5 g. with fat of known Issoglio value.

Theoretically this test, which depends mainly on the presence of the strongly odorous lower aldehydes, might be expected to bear some fairly close relationship to rancidity, as determined by taste and odour. Actually it suffers from the disadvantage that most fresh, sweet fats show a small oxidisability number which increases gradually on deterioration. The literature concerning the test is somewhat contradictory, some authors having obtained inconsistent results[163], while others[21, 175] have found it fairly satisfactory. Issoglio stated that normal fresh fats give oxidisability values of from 3 to 10, while a figure of 15 or over is considered indicative of rancidity. The figures obtained by Kerr conform roughly to these standards, though individual results were somewhat erratic.

Tests for the Carbonyl Group

For many years it has been known that rancid (oxidised) fats give reactions which indicate the presence of aldehydes and ketones, and the majority of the special tests for rancidity depend on this fact.

Carbonylic compounds in a fat may be estimated by determination of the amount of phenylhydrazine necessary to combine with them, or alternatively the solid condensation products may be filtered off and weighed[165]. A method of refining rancid oils by treatment with hydroxylamine, hydrazine, or semicarbazide, followed by filtration, has recently been patented[263, 278].

Stamm[275, 172] makes use of the colour reaction given by oxidised fats with diphenylcarbazide. This test, though useful with lard and beef-fat, is inapplicable to vegetable and marine animal oils[252].

The Kreis Test.

The Kreis test, originally described in 1902[173], has been for many years the most widely used chemical means for the detection of oxidative rancidity, and a considerable literature on the subject has been built up. To apply the test, 1 ml. of the oil or melted fat is shaken for one minute with 1 ml. of concentrated hydrochloric acid, followed by further shaking for a similar period with 1 ml. of a 0·1 per cent. solution of phloroglucinol in ether. If, on separation, the lower acid layer has acquired a red or pink colour, the fat is considered to be rancid, the depth of colour being a rough indication of the degree of rancidity.

Various modifications of the Kreis test have been proposed with the object of making it quantitative. Kerr[163], using 10 times the above quantities, diluted the sample with gradually increasing amounts of a non-reacting oil or solvent until a negative reaction was obtained, the amount of dilution necessary being considered a measure of the degree of rancidity. Holm and Greenbank[139] diluted the sample with petroleum ether until the colour obtained matched a methyl red standard. Other investigators have made use of potassium permanganate[240]. A series of six permanganate solutions, ranging in strength from 0·02 per cent. downwards each being 2·5 times weaker than its predecessor, enables an approximate estimate of intensity to be made with the aid of a simple block-comparator[191]. More satisfactory, however, than any of these methods is the use of the glass colour standards of the Lovibond tintometer, as suggested in the procedure recommended by the Committee on the Kreis test set up by the American Oil Chemists' Society[244]. A simple and satisfactory method employing this principle is as follows[190].

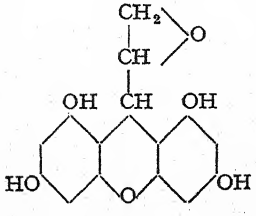
3 g. of oil are dissolved in 6 ml. of benzene in a small glass-stoppered cylinder and shaken with 3 ml. of concentrated hydrochoric acid for one minute. Five drops (0·1 ml.), of a 5 per cent. solution of phloroglucinol in alcohol are then added, the mixture shaken for one minute and separated by means of a (hand) centrifuge. Approximately 2 ml. of the clear aqueous layer is transferred to a 1 cm. glass cell and matched against the colour standards of the Lovibond tintometer (B.D.H. pattern with artificial light attachment). Alternatively, the solution contained in a small test-tube may be matched in a block comparator against the standards, placed in series with a blank solution. By use of a little yellow or blue in conjunction with the red slides, an accurate match can readily be obtained. Results may be expressed as the sum of the two colours, or 1 yellow unit may be considered as the equivalent of 0·2 red [244]. In the case of samples of fat giving colours deeper than about 10 units on the Lovibond scale, the quantity taken must be reduced, and the value obtained corrected to the normal weight. Blank determinations should be carried out without the phloroglucinol to allow for any charring effect of the acid on the oil, and without the oil to check the purity of the reagents.

When available, the Zeiss-Pulfrich photometer can be used to measure the colour developed in the Kreis test, and gives results of excellent reproducibility[176].

Purity of Reagents.—Diethyl peroxide in the ether interferes with the development and stability of the colour in the Kreis test[71], though to achieve the same result relatively large amounts of hydrogen peroxide appear to be necessary[289]. It is therefore advisable to purify ether containing peroxide by distillation from sodium hydroxide[191]. Hydrochloric acid containing nitrosyl chloride as an impurity will give a pink colour in the Kreis test, in the absence of rancidity[237], and the test is also unreliable in the presence of nitrite[148]. Each fresh batch of reagents should be tested before use.

A criticism of the Kreis test has been that a fat need not necessarily be rancid in order to give a red colouration with phloroglucinol. Crude cottonseed, maize and probably other vegetable oils, when perfectly fresh and sweet, give a strong positive reaction[163,273], though the colours developed are distinguishable by the spectro-photometer from those obtained from rancid oils[236]. The reaction in this case is probably due to non-glyceride constituents of the crude oil, which disappear during the process of refining with alkali. Essential oils and various aldehydes and ketones not likely to be present in rancid fats, are also said to give red colours with phloroglucinol[163,170]. These limitations do not appreciably detract from the value of the test, since fresh animal fats and refined vegetable oils, which include most of the cases likely to require examination, are free from reacting substances.

Mechanism of the Kreis Reaction.—Originally developed as an empirical test for rancidity, the theoretical basis of the Kreis reaction remained obscure until Powick[236], in the course of a systematic examination of 22 substances, considered as possible constituents of a rancid fat, found that only in the presence of both acrolein and a peroxide could a colour, spectroscopically identical with that given by rancid fats, be obtained. The oxidation product of acrolein responsible for the reaction was identified as epihydrin aldehyde, $CH_2\!-\!CH.CHO$ (O), and its red condensation product with phloroglucinol as



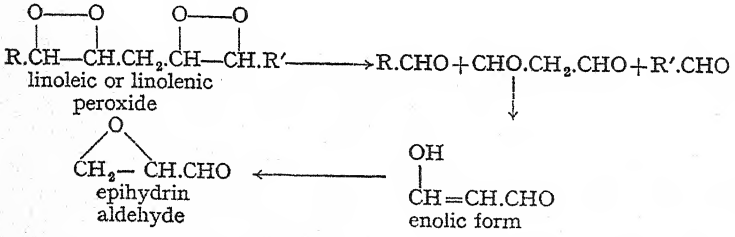
The free aldehyde could not be isolated in a pure state, because of its instability. Its diethyl acetal, however, was synthesised and shown to respond to the test. Powick therefore, suggested that the

Kreis reaction is due to the presence, in rancid fats, of an acetal of epihydrin aldehyde, a view which has subsequently found general acceptance.*

The mechanism of the formation of this substance is by no means clear. It cannot arise from the oxidation of glycerol, since fatty acids as well as glycerides respond to the test. Theoretically it could readily be derived from oxidative rupture of the outer two of a system of three conjugated double bonds, followed by loss of carbon dioxide, a scheme which has been used to explain the isolation of acrolein from the oxidation of linseed-oil and linolenic acid[258]. Unfortunately it is fairly well established that the linoleic and linolenic acids present in most oils and fats do not contain conjugated double bond systems. In any case, the Kreis reaction, in addition to being given by all unsaturated fats on oxidation, is also shown by oleic acid and its esters[289,17].

In order to overcome this difficulty Powick proposed a scheme whereby oxidative desaturation of oleic acid might result in formation of a conjugated tri-unsaturated acid, from which epihydrin and heptylic aldehydes could readily be derived. Slight variations of this scheme which have since been put forward, do not appear to possess any marked advantage over the original[223].

Actually it is not difficult to suggest a mechanism for the formation of epihydrin aldehyde from the $= CH . CH_2 . CH =$ grouping of ordinary linoleic and linolenic acids, but desaturation is necessary to produce it from oleic.



Pritzker and Jungkunz[240], assuming a 50 per cent. yield of epihydrin aldehyde from acrolein by the action of hydrogen peroxide, attempted to evaluate the colours obtained in the Kreis test as percentages of epihydrin aldehyde in the fat. They obtained figures ranging from 60 to 400 mg. of epihydrin aldehyde per 100 g. for several specimens of very rancid fats, and stated that those containing more than 10 mg. per 100 g. were inedible. This latter figure, according to their calculation, represents the decomposition of about 0·1 per cent. of the fat.

* It is, of course, also possible that the aldehyde does not exist, even as an acetal, in the fat, but is formed from some precursor, possibly a peroxide, under the influence of the acid used in the test.

Täufel and Russow[290] subsequently synthesised the diethyl and glycol acetals of epihydrin aldehyde from acrolein, and thence isolated the pure aldehyde as an unstable, volatile liquid, particularly

TABLE 28.—*Calibration of colour-standards for the Kreis test against epihydrin aldehyde*

(Täufel and Russow[290])

0·004%* alcoholic methyl red. (ml.)	0·01 N. sulphuric acid. (ml.)	Epihydrin aldehyde. (mg. per 100 ml.)
0·5	49·5	0·04
1·0	49·0	0·08
2·0	48·0	0·15
4·0	46·0	0·4
8·0	42·0	1·0
16·0	34·0	2·5
Potassium permanganate		
0·005 N.		5
0·01 N.		10
0·02 N.		20
0·04 N.		40

* The figure of 0·04 per cent. given in the original paper is a misprint.

susceptible to decomposition by alkali. By means of these acetals they calibrated a series of methyl red and permanganate standards against the colours given in the Kreis test (1 ml. of oil, 1 ml. of hydrochloric acid, 1 ml. of 0·1 per cent. phloroglucinol in ether) by known quantities of the pure aldehyde. From these figures (Table 28) the amount of epihydrin aldehyde present in a rancid fat can be ascertained directly.

The limit of sensitivity of this test was found to be at a concentration of one part of the aldehyde in about four million parts of fat. While agreeing with Powick that epihydrin aldehyde is probably present in a rancid fat as an acetal, from which it is liberated by action of the concentrated hydrochloric acid used in the test, Täufel and Russow consider that the other component is more likely to be a hydroxylated fatty acid than glycerol.

During the course of an exhaustive investigation of the test, these authors observed that in very rancid fats the accumulation of other aldehydes was liable to cause some interference by combination with phloroglucinol to produce feebly coloured condensation-products. Two modified techniques were therefore proposed. In the first[292], the fat is placed in a test-tube with the acid, and a plug of absorbent cotton, moistened with acid phloroglucinol solution, inserted in the neck. Rancidity is indicated by the

appearance, on warming, of a red colouration in the cotton. In the second method[288], volatile aldehydes are first removed by distillation in steam, after which the fat is cooled, concentrated hydrochloric acid added, and the liberated epihydrin aldehyde carried over by a stream of air or carbon dioxide into a mixture of equal volumes of a 0·1 per cent. alcoholic solution of phloroglucinol and concentrated hydrochloric acid. By increasing the quantity of fat and reducing the volume of phloroglucinol solution, it is possible to detect one part of aldehyde in 10 million parts of fat. An apparatus suitable for carrying out the test on small quantities of material has been described[291]. This is undoubtedly the most sensitive and most accurate form of the Kreis test, and as such should find use as a research method, but for routine use in the laboratory the simpler technique already described (page 98) is more suitable.

The Schiff Test

As early as 1899, Schiff's reaction for aldehydes was applied to the detection of rancidity in fats[267,33], but not until von Fellenberg's work 25 years later, did the method attain much prominence[87].

To prepare the reagent[240], 5g. of fuchsin are dissolved in 800 ml. of water, a solution of 5·4 g. of potassium metabisulphite in a little water and 100 ml. of N. hydrochloric acid added, and the volume made up to 1 litre. The solution is then decolourized by shaking with a little animal charcoal, filtered and stored in the dark. To make the test, 1 ml. of the oil or melted fat, diluted with an equal volume of petroleum ether, is shaken with 2 ml. of the reagent for one minute, and allowed to stand in the dark for 10 minutes. A colouration in the layer of either water or oil indicates rancidity.

A standard prepared by dilution of 0·25 ml. of 0·01 N. potassium permanganate to 1,000 ml. gave the same colour as an oil containing 20 mg. of acetaldehyde per litre. Samples showing weaker reactions than this were said not to taste rancid, while with stronger reactions the degree of rancidity was in general proportional to the content of aldehyde.

Von Fellenberg and Mundinger[88], have preferred the Schiff to the Kreis test as a criterion of oxidative rancidity, but Pritzker and Jungkunz[240], in a careful comparison of the two methods, failed to find any considerable difference between them. Actually the Schiff appears to be even more sensitive than the Kreis test, but does not so readily lend itself to quantitative use by colourimetric measurement, owing to the variable distribution of the colour between the two phases and the interface[17,190]. It is probable that the intensity of the colour produced is not, in any case, strictly proportional to the amount of aldehyde present.

For qualitative work the test is improved by use of chloroform in place of petroleum ether. In this case a milk-white or violet emulsion is produced which separates only slowly on standing.

Fat Aldehyde Value

Schibsted[264] has recently developed from the Schiff test a new technique which, because of its different application and performance, will be treated as a separate method. According to this method the fat in petroleum ether solution is shaken with a solution of rosaniline hydrochloride-sulphurous acid in 50 per cent. alcohol (which is red), and the colour produced in the petroleum ether layer compared with a cresol red standard at *p*H 8·3. Practical details are as follows :—

Reagents.—20 g. of rosaniline hydrochloride (dry basis) are dissolved in 1 litre of absolute alcohol, allowed to stand for several days and filtered. To 500 ml. of the clear solution is added an aqueous solution of 0·85 g. of sulphur dioxide (standardised by addition of 0·1 N. iodine in excess, and back titration with thiosulphate) and the volume made up to 1 litre. The reagent is then transferred to a number of small bottles, filled to the neck and stored in the dark. The petroleum ether used sholud be purified by shaking several times with concentrated sulphuric acid (until the acid is only faintly coloured), refluxed over 50 per cent. sodium hydroxide solution for two hours, and distilled from quicklime. A boiling range of from 50–100° C. is suitable.

Standard of Colour.—A 0·001 per cent. solution of cresol red is made up in borate buffer at *p*H 8·3. The buffer contains borax 7·5477 g., boric acid 7·5044 g., and sodium chloride 1·7696 g. in 1 litre. This colour is taken as 100 units on an arbitrary scale.

The Method.—The oil or fat is dissolved in petroleum ether to such a concentration that the colour obtained lies between 50 and 200 units. 25 ml. of this solution and 5 ml. of the reagent are pipetted into a test-tube (22 × 175 mm.), which is closed with a tin-foil-covered rubber stopper. The tube is then rotated for four minutes at about 30 rotations per minute about an axis perpendicular to its length. After standing for a short time about 20 ml. of the coloured petroleum ether solution are transferred to a clean test-tube, closed by the same stopper and allowed to stand for one or two hours. after which the colour is matched against the cresol red standard in a Duboscq or similar colorimeter. The fat aldehyde value, which is the colour, in arbitrary units, which would be developed by a 0·1 per cent. solution of the fat under the conditions of the test, is given by the expression

$$V = \frac{100 \times D_s \times 0{\cdot}1}{D_t \times C}$$

where D_s and D_t are the depths of colour standard and test solution matched in the colorimeter, and C the concentration (as per cent.) of the fat solution tested. Lea[190] has slightly simplified the test by calibrating the cresol red standard against the Lovibond colour glasses (4·75 red, 1·1 blue in 1 cm. cell), so that the experimental solutions can be matched directly in the tintometer.

In carrying out the test it is advisable to follow closely the conditions specified, since Schibsted's paper indicates that the depth of colour obtained from a given specimen of fat is influenced by the concentrations of alcohol, sulphur dioxide and hydrochloric acid employed, by the proportions of the reagent and fat solution, and by the shape of container, manner of shaking, and time and conditions of standing, all of these variables having been adjusted as far as possible for maximal development of colour.

In spite of its apparent complication, the method is not difficult to carry out as a routine test, and is said to be about 20 times as sensitive as the ordinary Schiff technique. Simple aldehydes such as heptaldehyde do not develop any colour in the petroleum ether phase, the test apparently being specific for aldehydes of high molecular weight, whose condensation-products with rosaniline hydrochloride are soluble in petroleum ether. The substances reacting in rancid fats are therefore presumably the glyceride-aldehyde residues which remain after splitting off of the simple aldehydes. Schibsted further states that samples of oleic and linoleic acids which had quite a tallowy odour, and gave a positive reaction in the ordinary Schiff test, failed to give any colour with the new reagent.

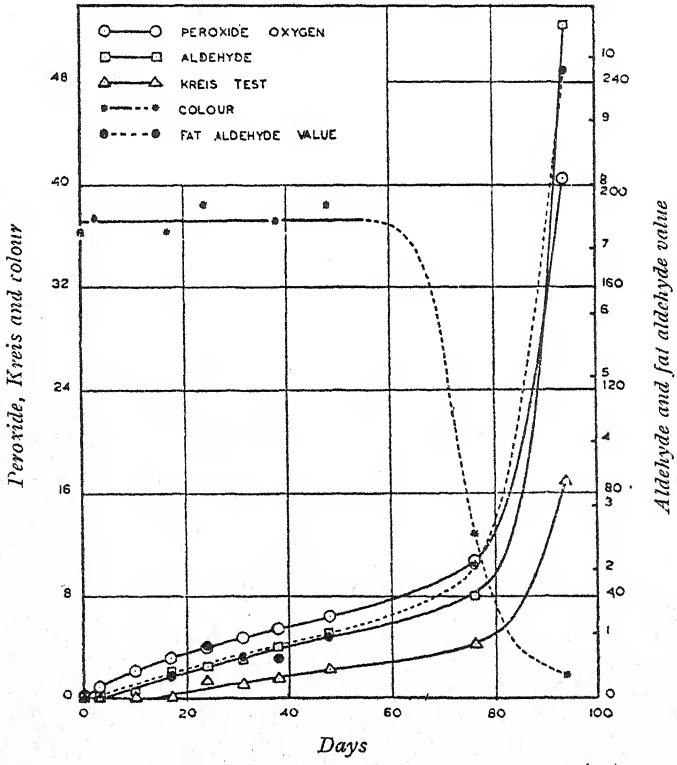


FIG. 14—*Oxidation of butter-fat in lamplight.* (Lea [190])

Very little work on this test has yet been published. The original paper gives a short table of values ranging from 0·5 to 20 for non-rancid fats, and from 10 to 360 for rancid specimens, the overlap being due to butter-fat, which invariably shows perceptible rancidity at very low values of the chemical tests. Lea[190] has

compared the increase in fat-aldehyde value with development of peroxide, Kreis test, aldehyde-content and colour in oxidising butter-fat (Fig. 14), and Barnicoat[18] has commented favourably on its use as a means for investigating " toppiness " in butter.

Estimation of Aldehydes with Bisulphite

Aldehydes of medium molecular weight are usually considered to be the substances most intimately concerned in the production of the objectionable odours and flavours of oxidised fats (page 112). Lea has recently devised a method whereby the minute quantities of aldehydes present in a rancid fat can be estimated directly by titration with sodium bisulphite[190]. The test is applied as follows :—

Reagent.—A bisulphite solution is prepared by dissolving 66 g. of anhydrous sodium carbonate in approximately 450 ml. of water, saturating with sulphur dioxide gas and making up to 500 ml. This stock solution is stored in the dark and a portion diluted 10 times when required.

Method.—Approximately 1 g. of the fat is weighed into a glass-stoppered bottle of roughly 70 ml. capacity, dissolved in 2 ml. of A.R. benzene, 20 ml. of bisulphite solution added and the mixture shaken at about 240 cycles per minute for one hour at 20° C. in the absence of light. If the temperature is below 20° C., the time should be increased by 10 per cent. for each degree. The emulsion is then transferred to a tube, centrifuged for about five minutes and 15 ml. of the clear aqueous layer pipetted into a 150 ml. Erlenmeyer flask. Iodine (non-standard) is run in, first N., then 0·05 N and finally 0·002 N., until the excess of bisulphite has been destroyed. The end-point is adjusted to a definite depth of tint in the presence of starch. 3 g. of A.R. sodium bicarbonate are now added and the sulphite, which is thereby liberated from combination with the aldehyde, titrated with standard 0·002 N. iodine solution until the same end-point is again reached. Towards the end of the titration the colour produced by the latest addition of iodine tends to fade more and more slowly, but the true endpoint is definte and can easily be determined. In this, as in the peroxide estimation, it has been found preferable, though not essential, to titrate with the aid of a day-light lamp in a darkened room. Correction is made for a blank determination (*ca.* 0·2 ml.) carried out concurrently without the fat. The aldehyde-content is expressed either as ml. 0·002 N. iodine per gram of fat, or multiplied by 28 as parts (CO) per million of fat.

Heptaldehyde added to fresh cottonseed-oil could be recovered quantitatively by shaking for 60 minutes at 20° C. The apparent aldehyde-content of oxidised fats showed some tendency to increase slightly with the time of shaking. This may be due either to slow combination of less reactive aldehydes of high molecular weight, or, more probably, to the gradual decomposition of peroxide with the production of further quantities of aldehyde. Fresh beef and butter fats, lard and cottonseed-oil, gave values of 0·0, 0·0, 0·1 and 0·1 ml. 0·002 N. iodine per gram respectively, while moderately rancid samples of the same fats showed values ranging up to 10·5 ml per gram (Fig. 14). Preliminary results with cottonseed-oil seemed to indicate that in this case the estimation of aldehyde might follow rancidity, as measured by taste and odour, more closely than either the peroxide or the Kreis tests[190], but here, even more than for the Schibsted test, further work is necessary.

Data recently published[176] indicate that the aldehyde, peroxide and Issoglio values of butter-fat exposed to light increase in proportion to one another, but not to the Kreis test. Hamilton and Olcott[124] by this method have estimated the aldehydes produced during the oxidation of methyl oleate and of oleic acid.

Estimation of Peroxide Oxygen

Every theory of the autoxidation of unsaturated fatty acids postulates the primary formation of substances possessing peroxide properties. Rancid (oxidised) fats in the presence of a peroxidase (separated milk, blood, or plant-juice) give characteristic peroxide colour reactions with guaiacum, benzidene, p-phenylenediamine hydrochloride or p-phenylenediamine hydrochloride and α-napthol (indophenol reaction). Decolourised (reduced) indicators of graded oxidation-reduction potential revert to the coloured form when added to a fat, according to the stage of incipient oxidation reached by the sample[35]. Oxidation of titanous or ferrous (page 111) salts can also be used for the estimation of peroxide. Attention, however, has been largely concentrated on the iodimetric method.

The Iodimetric Method

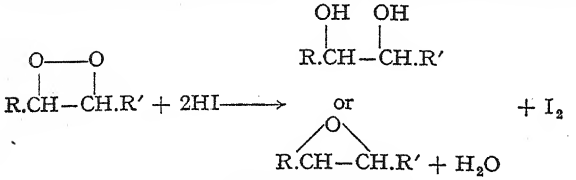
Numerous qualitative forms of the iodimetric method were used in early work[130, 236, 137] and in a few cases some attempt was made to estimate the iodine liberated either colorimetrically[321], or by titration with thiosulphate[70]. The quantity of peroxide found was always small, and the liberation of iodine slow and incomplete. Thus, Holm and Greenbank[139] observed that oxidised butter-fat still slowly liberated iodine even after standing for 24 hours with aqueous potassium iodide solution, while the total quantity measured corresponded only to some 3–4 per cent. of the oxygen absorbed. They concluded that " neither iodine value nor iodine liberation is sensitive enough to detect small changes through oxidation."

Traces of reactive peroxides certainly exist in oxidised fats, or are produced when the fat is treated with water or aqueous alcohol. Hydrogen peroxide can readily be detected, particularly after exposure to light, by its action in fogging a photographic plate placed a little distance above the sample[281], and traces of peracids may be formed as intermediate products of the autoxidation of aldehydes produced by the decomposition of the fat peroxides.

$$R.CHO + O_2 \longrightarrow R.CO.O.OH \text{ (Peracid)}$$
$$R.CO.O.OH + R.CHO \longrightarrow 2R.COOH$$

These substances immediately liberate iodine from acidified potassium iodide solution. The majority of the peroxides present in oxidised fats, however, behave like the di-substituted hydrogen peroxides (e.g., benzoyl and diethyl peroxides), in that they are reduced so slowly by ordinary acidified aqueous potassium iodide solution that estimation by this means is impossible. They are decomposed quantitatively by a solution of potassium iodide in

hot glacial acetic acid[102, 191], probably according to the equation



In the method described below this reagent is used with the addition of sufficient chloroform or carbon tetrachloride to form a single phase system with the fat. Since a solution of potassium iodide in glacial acetic acid oxidises rapidly in the air, particularly on exposure to light, precautions are taken against oxidation of the reagent during the determination.

Method (*Lea*).—1 g. of the oil or fat is weighed into a numbered and tared pyrex test-tube, approximately 17 mm. in diameter. Powdered potassium iodide (*ca.* lg.) is added, followed by 20 ml. of a mixture of glacial acetic acid and chloroform or carbon tetrachloride (2 : 1 by volume). A rubber stopper, bored with one hole, is fitted, and nitrogen or carbon dioxide passed into the air-space above the liquid for about one minute to displace most of the air. The tube is then heated in an inclined position over a small flame applied to its lower end, a finger resting lightly over the hole in the stopper and the tube rotating slowly to prevent cracking. As soon as the solution is bubbling fairly freely, the tube is plunged into a boiling water-bath. The liquid boils smoothly, chloroform vapour expelling any remaining traces of air. When vapour begins to issue from the tube, as felt by condensation on the stopper, the finger is removed and a glass plug forced in. This operation is preferably carried out with the liquid frothing nearly to the top of the tube. In the presence of non-fatty material, the tube is now shaken for a few seconds to ensure complete extraction of the fat. Otherwise it is simply cooled under the tap and the contents poured into about 30 ml. of water. The tube is rinsed out with a little water and the free iodine titrated with 0·002 N. sodium thiosulphate, adding starch solution as the end-point is approached. 1 ml. of 0·002 N. thiosulphate per gram is equivalent to 1 millimole, or 2 milli-equivalents of peroxide, 16 mg. of *active* oxygen or 32 mg. of total peroxide oxygen per kilogram of fat.

It is of advantage to carry out the whole operation by artificial light (titrating with a "daylight" lamp), since by this means possible oxidation of the fat or reagents during handling is eliminated. Under these conditions, reagents of A.R. quality and perfectly fresh fats give zero values,* and the end-point (except in the case of deeply coloured samples) is easily readable to 0·1 ml. As can be seen from the data published in the original papers[192, 191], the degree of reproducibility obtainable is of a fairly high order.

A simplified method which dispenses with the use of inert gas and artificial light, may be employed for the routine testing of fats and oils. Naturally, exposure to direct sunlight or skylight during the determination should be avoided.

Simplified Method.—1 g. of the oil or fat is weighed into a pyrex test-tube, approximately 1 g. of powdered potassium iodide and 20 ml. of glacial acetic

* A blank titration may be due to the presence of copper in the distilled water.

acid-carbon tetrachloride (or chloroform) mixture (2 : 1 by volume) added, and the liquid heated to boiling over a small flame impinging on the bottom of the tube. Boiling may be continued for half a minute, the heavy vapour of the solvent minimising the diffusion of oxygen back into the tube. The tube is then cooled under the tap, the contents poured into 30 ml. of water and titrated with 0·002 N. thiosulphate. Low peroxide-values should be corrected for a blank on the reagents.

The 0·002 N. thiosulphate solution, which may with advantage contain 0·02 g. of sodium carbonate per litre, is prepared directly, or by dilution of a 0·1 N. solution with water boiled free from carbon dioxide. It is stored in a syphon-bottle fitted with a soda-lime guard-tube and protected from light. At intervals of about a week it is standardised, in the presence of potassium iodide and a little acid, against a dilute iodine solution, prepared directly from the pure solid or by dilution from a 0·1 N. stock solution.

1 g. of fat is the quantity employed when, as is usually the case, the samples examined are in the early stages of oxidation. 0·5 or even 0·1 g. is sufficient for more highly oxidised oils[188]. In a particular piece of work the amount of fat used should be standardised, since a certain loss of iodine through reabsorption always occurs, and this is greater the larger the sample and the higher its unsaturation and degree of oxidation. For slightly oxidised fats of relatively low iodine-value, the loss is small[191]. Several modifications of the peroxide test have since been proposed[284, 311], usually with the object of simplifying the method for the routine testing of fats and oils in the laboratory. Wheeler's technique, which has been used extensively in the United States, is carried out as follows :—

Method (Wheeler).[311] — 3–10 g. of oil are dissolved in 50 ml. of a mixture of glacial acetic acid and chloroform (3 : 2 by volume) and 1 ml. of saturated potassium iodide solution added. The mixture is stirred by giving a rotary motion to the flask. Exactly one minute after addition of the potassium iodide, 100 ml. of water are added and the liberated iodine titrated with 0·1 or 0·01 N. sodium thiosulphate, depending on the amount of iodine liberated. The end-point is obtained by the use of starch as an indicator. Vigorous shaking is necessary to remove the last traces of iodine from the layer of chloroform. If W g. of oil require T ml. of thiosulphate of normality N, the peroxide-content, expressed as millimoles of peroxide per kilogram* of oil, is given by

$$\mathrm{M} = \frac{T \times N \times 500}{W}$$

Taffel and Revis[285] have also described two procedures for the estimation of peroxide. In these, solid barium iodide or 50 per cent. potassium iodide is used with glacial acetic acid but without the addition of chloroform or carbon tetrachloride. Contact between fat and reagent is assisted in both cases by alternating two-minute

* This unit is exactly equivalent to the " ml. of 0·002 N. thiosulphate per gram " used in English publications.

periods of shaking and standing. The procedure at room temperature which is recommended for slightly rancid samples is inapplicable to solid fats, and appears to give rather low results with oils, while the presence of 10 g. of fat on the surface during titration is a disadvantage in both methods. That carried out at 100°C. gives a high and variable blank determination, owing to incomplete exclusion of air. In this respect the method has recently been improved by Giles[99], but results are then liable to be somewhat low, possibly owing to insufficient mixing of fat and reagent.

Taffel and Revis point out that oils which have been blown with air or oxygen at high temperatures *e.g.*, 120°C.) contain in addition to peroxides which can be estimated by the methods already described, a certain proportion of oxy-compounds, reducible with more difficulty, which only liberate iodine when boiled with glacial acetic acid and barium iodide (120°C.) for half an hour. In oils blown at 170°C., practically all of the reducible oxygen is in this form. Oxy-compounds, reducible with difficulty, are not, however, formed to any considerable extent in oils becoming rancid at ordinary temperatures.

TABLE 29.—*A comparison of several procedures for the iodimetric estimation of peroxide.*

(Lea[198])

Method.	Weight of fat.(gr.)	Temperature. (°C.)	Cotton-seed oil.	Leaf-lard.	Butter-fat.
Lea (*a*)* KI	1	60	18·0	16·3	20·1
BaI_2	1	60	15·8	15·2	22·5
(*b*)† KI	1	100	15·6	12·5	18·2
BaI_2	1	100	16·9	13·3	23·9
(*c*)† KI	1	120	14·3	11·5	18·8
BaI_2	1	120	16·3	13·9	24·9
Wheeler KI	5	room	12·1	13·2	16·1
Giles KI	8	100	9·0	9·3	16·4
Taffel and Revis KI	10	100	—	15·7	—
BaI_2	10	100	15·6	14·2	—

* Solvent, 20 ml. of acetic acid-chloroform.
† Solvent, 20 ml. of acetic acid.

As can be seen from Table 29, the peroxide-value obtained from a given specimen of fat, varies considerably according to the technique of estimation employed, owing both to the non-uniform reactivity of the peroxides present and to different degrees of reabsorption of the liberated iodine at the unsaturated centres. This latter factor cannot be dismissed, as both Wheeler and Taffel and Revis

have suggested, on the result of a trial estimation on unoxidised fat in the presence of added iodine or even of benzoyl peroxide, since the loss of iodine in both cases appears to be less than when fat peroxide is decomposed *in situ*[191]. For the majority of purposes, however, the absolute value obtained for the peroxide-content is of less importance than the attainment of consistent and reproducible results.

Wheeler's and Taffel and Revis' room-temperature methods tend to give lower values than Lea's original technique (a). Taffel and Revis' determination at 100°C. may give higher or lower values, according to the nature of the peroxides and unsaturated acids present. Blank titrations, for which the values given in the table have been corrected, were (a) 0·0, 0·45; (b) 0·12; 0·62 and (c) 0·30, 1·05, for the potassium and barium salts respectively.

In case of highly oxidised fats, loss of iodine by reabsorption can be minimised by the use of smaller amounts of fat, which in effect reduces the concentration of both reactants. Since the end-point in the titration can be determined to 0·1 ml., it is of advantage in accurate work (but unnecessary for comparative purposes) to adjust the amount of fat to keep the titration always below 10 ml.[176]

Peroxide values (method (*a*)) determined on progressively diminishing amounts of fat have been found as follows—

Butter fat (g.)[176] ..	1·004	—	0·512	0·203	0·112	0·050
peroxide (ml. per g.) ..	36·9	—	44·4	51·9	58·7	56·3
Pig's leaf fat (g.)[198] ..	1·0	0·8	0·6	0·5	0·2	0·1
peroxide (ml. per g.) ..	70·3	70·8	72·7	74·0	79·4	81·9

Estimation of Peroxide in the Presence of Non-Fatty Material.—To obtain an accurate record of the progress of oxidative rancidity in a foodstuff, the fat should be extracted from the tissue and examined separately. It is, however, sometimes possible in the case of foods of high fat-content to save time and labour by carrying out a direct determination of peroxide oxygen on the material itself. This method has been applied successfully to the adipose tissue of various meats[179–183], of which the fat-content usually exceeds 80 per cent., and to butter. The material, if reduced to a sufficiently fine state of subdivision, is thoroughly extracted by the boiling solvent employed in the test. Inevitably, a certain proportion of the iodine liberated is lost through chemical combination with or absorption by the protein, but this, in the cases cited, is not large, and the values obtained are quite suitable for comparison of the effect of different storage conditions on keeping properties. Data comparing the two methods in the case of pig fat have recently been reported (Lea, C. H. Report of the Food Investigation Board for the Year 1937). The end-point of the titration is less sharp than when pure fats are used.

This procedure cannot be extended, except in a rough way, to materials containing large amounts of non-fatty substances. The

TABLE 30.—*Effect of non-fatty material on the peroxide estimation,* (Lea[198])

Chicken's skin (9–21% fat).			Dried milk (28% fat).			
Direct determination. (1 g.)	Calculated for fat. (1 g.)	Observed on fat. (1 g.)	Odour and Flavour.	Direct determination. (1 g.)	Calculated for fat. (1 g.)	Observed on fat (1 g.)
0·0	0	2	Fresh	0·0	0	1·0
0·0	0	2	Slightly tallowy	0·0	0	2·7
0·0	0	5	Tallowy	0·0	0	3·7
0·15	1	6	Very tallowy	0·0	0	6·5
0·3	3	12	Very tallowy	0·35	1·2	8·2
1·25	6	19				
2·0	22	59				

data quoted in Table 30 are for (a) young chickens stored at 0°C.[186] and (b) full-cream dried milk after exposure to skylight on a winter afternoon for 0, 15, 30, 60 and 120 minutes.

Estimation of Peroxide by Ferrous Salts

Peroxides in hydrocarbon oils have been estimated by oxidation of ferrous to ferric iron, and in its latest form[322] this method might perhaps prove suitable for application to fats. Such a procedure would possess an advantage over the iodimetric technique, in that loss by reabsorption of iodine would be eliminated. Ferrous sulphate, however, is less easily oxidised than acid potassium iodide[323], and only the more reactive peroxides would be estimated in this way. As described for hydrocarbon oils, the determination is carried out as follows.

The reagent is prepared by dissolving 5 g. of ammonium thiocyanate and 5 ml. of 6 N. sulphuric acid in 1 litre of absolute methyl alcohol, and then saturating the solution with pure ferrous ammonium sulphate by shaking with the powdered solid. To 10 ml. of this solution a quantity of the sample equivalent to 0·00002–0·0002 mole of ferric salt is added. With some hydrocarbon peroxides the colour reaches maximal intensity in a few seconds. In the case of fats it would probably be advisable to heat the solution to just below boiling point for 4–5 minutes, as prescribed for the less reactive peroxides. The colour produced is compared in a Duboscq colorimeter with standards prepared by adding ammonium thiocyanate and sulphuric acid in the proportions used in the reagent to solutions of ferric chloride in absolute methyl alcohol. These standards should be freshly prepared each day and stabilised by addition of a small amount of a reactive peroxide.

Extraction of Fat for Examination

Special care is necessary during the extraction of fat from a foodstuff for the estimation of peroxide, if further oxidation during the process or decomposition of the peroxide already present is to be prevented. Details of the method employed must, of course, depend on the particular material under investigation, but the following general notes may serve as a rough guide to the procedure adopted.

Materials containing more than a few per cent. of water should be dried before extraction. Small samples can be dried rapidly and safely at ordinary temperatures by exposing the material in thin layers, over calcium chloride, to the vacuum produced by an oil-pump. When drying becomes slow, owing to the formation of a crust, the material is stirred or powdered and replaced in a second desiccator over fresh calcium chloride. The latter should be redried before it shows any signs of moistening. Substances in which frothing is not troublesome may be dried directly in a round-bottomed flask under vacuum at room temperature or at 50°C.

Extraction is preferably carried out with peroxide-free ether or with petroleum ether in a hot-extraction apparatus. This consists of a flask, having a long wide neck and fitted with a ground-in double-surface condenser. The thimble containing the dried material is suspended in the neck of the flask, where it is surrounded by the vapour of the boiling solvent. After extraction the solvent is partly distilled off and the remainder removed under vacuum (round-bottomed flask) at a temperature not exceeding 50°C. An ether-soluble fraction, amounting to only 0·2–0·4 per cent., can be recovered unoxidised in this way from muscle containing 75 per cent. of water[186].

SECTION 3.—THE RELATION BETWEEN THE CHEMICAL TESTS AND RANCIDITY

Products Responsible for Rancid Odour and Flavour

The degree of correlation with rancidity, as measured by odour and flavour, to be expected from a chemical test which estimates some individual compound or group of compounds out of the considerable number present in an oxidised fat, obviously depends on which of these substances are actually responsible for the unpleasant odour and taste.

Atmospheric oxidation of unsaturated fats has been shown to give rise to peroxy-, oxy-, hydroxy- and keto-glycerides or acids, hydrogen peroxide, carbon monoxide and carbon dioxide, dicarboxylic acids, acrolein, epihydrin aldehyde, glyceride-aldehydes and acids, and almost the complete series of volatile aliphatic monobasic acids and aldehydes from formic to nonoic. Early work[259,260] suggested that aldehydes of medium molecular weight, particularly heptaldehyde and nonaldehyde, and in less degree hexaldehyde and butaldehyde, are the compounds responsible for the odour and flavour of rancid (oxidised) fats. Powick[236], from

direct examination, concluded that none of the following substances is capable of contributing appreciably, if at all, to the odour,—hydroxy-, dihydroxy- and diketostearic, formic, acetic, butyric, caproic, heptylic, caprylic, nonoic, azelaic, and acrylic acids, formaldehyde, acetaldehyde, butyric aldehyde, acrylic aldehyde (acrolein), crotonic aldehyde, methyl glyoxal and dihydroxy acetone. On theoretical grounds, ketoxystearic acid and azelaic semi aldehyde were also eliminated. He decided that the odours of heptylic and, in less degree, nonoic aldehyde, either alone or in the presence of fresh fats, are sufficiently suggestive of the odour of rancidity to establish the reasonableness of Scala's contention that these are the substances primarily responsible for rancidity. A study of the literature indicates that this opinion has been almost universally accepted.

The odour of heptylic aldehyde can easily be detected when this substance is dissolved in paraffin at a concentration of 1 or 2 parts per million[17], and the same is true for solutions in fresh cotton-seed oil. In neither case does the odour, though certainly suggestive of rancidity, faithfully reproduce that of an oxidised, "tallowy" fat. The odour of the mixture of lower fatty acids obtained as a by-product in the acetone-permanganate oxidation of unsaturated glycerides[133] more closely resembles that of an excessively rancid fat. There is therefore some reason for the belief that volatile fatty acids contribute to rancidity, though on the basis of Powick's results they are probably of less importance than the corresponding aldehydes. Still other substances may perhaps assist in the production of the burning after-taste which is one of the usual symptoms of this form of spoilage. In any case the variation in the quality of the odour and taste observed in different samples of fat makes it improbable that heptylic and nonoic aldehydes are the only substances involved in the production of oxidative rancidity, though they may be the most important.

Limitations of the Chemical Tests

Since the Kreis test was for many years the method most widely used for the estimation of rancidity, much of the controversy concerning the value of the chemical tests in general has centred round its use. Numerous investigators[240, 289] have found the Kreis test a valuable and sensitive method for the identification of oxidative rancidity. Kerr[163, 164] stated that the depth of colour obtained in the test is roughly proportional to the degree of rancidity, and classed fats reacting at a higher dilution than 1 in 20 as definitely rancid. Holm and Greenbank[139], working on the oxidation of butter-fat and lard at 95° C., found that every sample in which appreciable oxidation had occurred gave a positive Kreis test, but the intensity of the colour produced bore no direct relation to the degree of rancidity, as measured by taste and odour. The colour was, however, proportional to the amount of oxygen

which had been absorbed by the fat. Triebold[297], on the contrary, found that the amount of oxygen which had been absorbed was roughly proportional to the log of the intensity of the colour. Numbers of other observers reported that the Kreis test could not be used as a true measure of rancidity[53, 58]. A special committee, set up by the American Oil Chemists Society, carried out co-operative tests between a number of laboratories and recommended that, because of the difficulty experienced in obtaining reproducible results, and because of its somewhat uncertain relation to quality in edible fats and oils, the Kreis test should not be added to the official methods of the Society[244]. The German Fat Analysis Commission, even more definite in its condemnation, stated that none of the colour reactions used as tests could be considered sufficiently reliable for adoption as standard methods, and that taste and odour remained the only reliable criteria of rancidity[58, 59]. The situation with regard to others of the chemical tests has been somewhat similar. Recent work has done something to dispel this confusion.

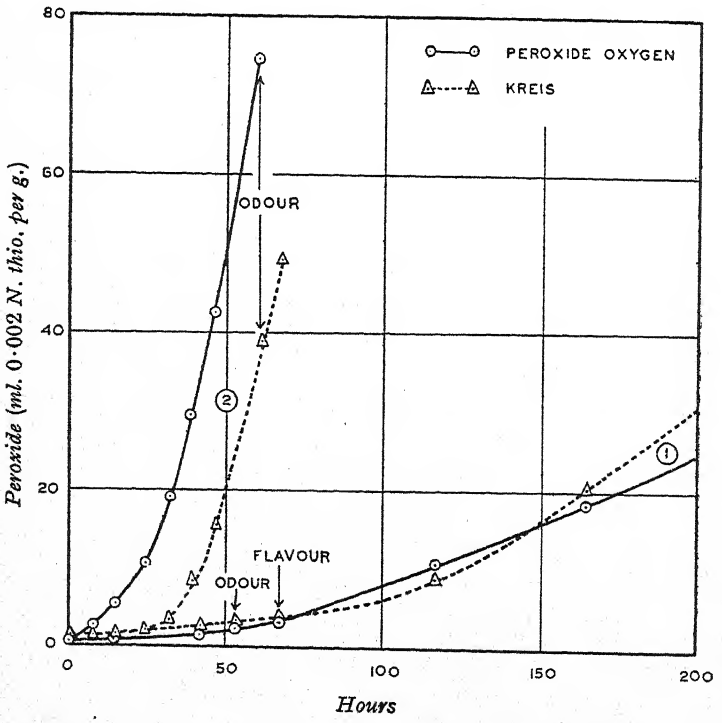


FIG. 15.—*Influence of composition of the fat on the values of the chemical tests at the appearance of rancidity.* (1) *Methyl oleate,* (2) *Methyl oleate with linoleate* (1 : 2). *Exposed to lamp light at* 26° *C.* (Barnicoat[17])

Effect of Composition of the Fat

Barnicoat[17] has investigated the relation between the composition of the fat and the conditions of storage, on the one hand, and the values of the peroxide and Kreis tests at which rancidity first becomes perceptible. His results for several animal fats showed that, under a given set of conditions, the values of the chemical tests at which rancidity could first be detected decreased with the content of acids less saturated than oleic. This conclusion was confirmed by experiments in which (*a*) methyl oleate and (*b*) a mixture of methyl oleate and linoleate in the proportion of approximately 1 : 2, were allowed to oxidise at 26° C. in the light from an ordinary tungsten lamp. The results (Fig. 15) show that, under strictly comparable conditions, the linoleate mixture oxidised the more rapidly of the two but a rancid odour and flavour appeared much earlier in relation to the chemical tests in the oleate. Table 31 gives the peroxide and Kreis values at which the sample could first be distinguished with certainty from its control by three observers.

Effect of Temperature

When samples of the mixed esters were heated at 103° C., oxidation proceeded at a greatly increased rate, and the oil then acquired a powerful " oxidised fat " odour and a rancid, acrid flavour at values of both tests far below those previously attained at 26° C., without the production of perceptible rancidity. The effect of heat on the values at which rancidity appeared in the case of methyl oleate was much less marked.

TABLE 31.—*Effect of the presence of acids less saturated than oleic on the values of the chemical tests at the appearance of rancidity.* (Barnicoat[17])

Ester	Rancid odour		Rancid flavour	
	Peroxide (ml. 0·002 N. thio. per g.)	Kreis (units)	Peroxide (ml. 0·002 N. thio. per g.)	Kreis (units)
Methyl oleate (*a*)	2·2	3·0	3·0	4·0
Methyl oleate (*b*)	0·7	2·8	1·2	3·8
Methyl oleate with methyl linoleate (1 : 2)	75	39	>82	>49

Effect of Light

A somewhat similar behaviour was observed with regard to the action of light (Table 32). Exposure to strong light (e.g., sunlight) not only greatly accelerated oxidation, but caused the objectionable odour and flavour of oxidised fat to become apparent at much lower peroxide-oxygen and Kreis values than when diffused daylight,

TABLE 32.—*Effect of light on the values of the chemical tests at the appearance of rancidity* (Barnicoat[17])

Fat	Acids less saturated than oleic (%)	Light	Mean temperature (° C.)	Rancid odour		Rancid flavour	
				Peroxide (ml. 0·002 N. thio. per g.)	Kreis (units)	Peroxide (ml. 0·002 N. thio. per g.)	Kreis (units)
Beef kidney ..	2·6	Sunlight	35	0·7	—	—	—
„ „ ..		Daylight	17	1·5	—	—	—
„ „ ..		Lamp	27	2	—	—	—
Beef external..	4·5	Sunlight	26	2·4, 3·2	0·7, 1·3	5	4
„ „ ..		Daylight	25	13, 17	18, 27	29	59
„ „ ..		Daylight	20	9, 16	7, 20	20	32
Lard	8·9	Sunlight	35	8	11	8	11
„		Lamp	27	43	65	29	47

artificial light or darkness was used. The heat of the sunlight was certainly not responsible for this effect, though it probably contributed slightly to it. This observation has recently been confirmed[51, 50], in the case of oils exposed to air in daylight and in darkness (or protected from chemically active rays of light). Much higher peroxide values could be reached without production of a rancid odour in darkness than in daylight.

Holm and Greenbank[140] had previously observed that oleic acid, stirred in an atmosphere of oxygen at 95° C., gave a strong tallowy odour on slight absorption of oxygen, while linoleic showed only a faint tallowy odour after absorption of relatively large amounts of oxygen, and linolenic acid, no odour at all. They therefore put forward the suggestion that the substances responsible for the rancid odour and taste of oxidised fats are products of the degradation of oleic rather than of linoleic or linolenic acids. These latter acids would tend to oxidise selectively and give strong chemical tests for oxidation before appreciable quantities of oleic acid were attacked. The criterion for the development of rancidity at low values of the chemical tests would therefore be a low content of acids less saturated than oleic. Barnicoat's results, obtained at room temperature, appear to support this conclusion.

Stability of the Peroxide

Peroxides are formed as the first step in the process of oxidation, and subsequently undergo decomposition to produce both the substances responsible for the chemical tests and those responsible for rancidity. The peroxide-content measured must therefore correspond to the excess of the quantity formed over that decomposed. The same is true in less marked degree for some of the other tests, since the substances responsible for them may also undergo further change. Negative or quite low Kreis and Issoglio tests, for example, are occasionally given by excessively rancid fats.

Fat peroxides vary considerably in stability, according to their structure. As already pointed out (page 91), one of the peroxide groups of fully oxidised β-elaeostearin is sufficiently stable to pass unchanged through a series of chemical reactions, and even survives distillation *in vacuo*. Linolenic acid, its esters and triglyceride have been autoxidised at ordinary temperatures to saturation of 50 per cent. or more of their double bonds, with very little secondary decomposition of the peroxide (Table 27), in spite of the presence of a cobalt catalyst which tends to favour such decomposition. On the other hand, the peroxide of oleic acid appears to be much less stable and commences to break up immediately. High temperatures and exposure to strong light favour decomposition of the peroxide.

At low temperatures decomposition is retarded. Samples of corn and cottonseed oils blown with air, until they contained peroxide equivalent to 5–60 ml. 0·002 N. thiosulphate per g., showed no change when stored in the absence of oxygen at 5° C. for six

months[166]. On the other hand, peroxide equivalent to 200 ml. per g. was completely destroyed by heating for seven days in a sealed tube at 85° C.[6] Coe and Leclerc[51], bubbling oxygen through similar oils at room temperature in the absence of light,

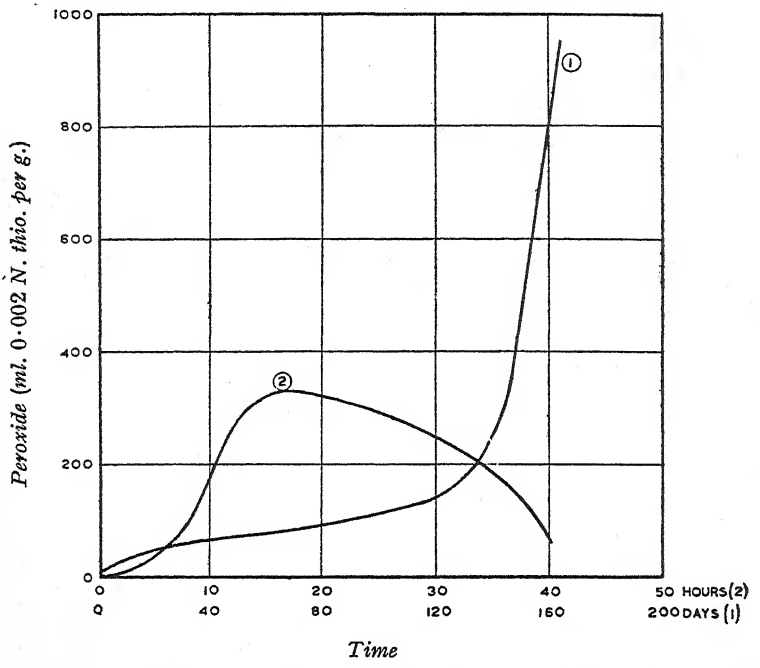


FIG. 16.—*Accumulation of peroxide in cottonseed-oil, blown with oxygen,* (1) *at room temperature* (Coe and Le Clerc[51]), (2) *at* 100° *C.* (Wheeler[311])

recorded peroxide-values up to 950 ml. per g. without observing any signs of falling off in the rate of accumulation. At 100° C. the peroxide-content passed through a maximum at about 300 ml. per g., and then gradually decreased (Fig. 16), in spite of continued absorption of oxygen and fall in iodine-value[311].

The temperature necessary for *rapid* decomposition of fat peroxide is, however, fairly high. Roschen and Newton[253] found that samples of lard, aerated at 80–90° C. until they became rancid (peroxide content 32 ml. per g.), or highly rancid (91ml. per g.), showed no appreciable loss of peroxide when blown with steam for three hours at 100° C. The resulting lards were odourless, bland to the taste and palatable, owing to the removal of the volatile products of decomposition responsible for rancidity. Obviously the peroxides themselves have no part in the production of the rancid flavour. Samples blown with steam for a further three hours at 200° C. were also odourless and the flavour, though flat, was not rancid. The

peroxides, however, had completely disappeared (0·1 and 0·9 ml.). The temperature of decomposition, as indicated by the evolution of volatile products, was probably in the neighbourhood of 140°-160° C. This observation agrees well with the fact that peroxides, as measured by the ordinary methods of estimation, no longer accumulate in oils exposed to oxygen at 170° C., being presumably decomposed as rapidly as they are formed[285].

Relation between the Chemical Tests

Composition of the fat, temperature of storage and exposure to light, in addition to affecting the values of the chemical tests at which rancidity appears, also influence in some degree the relative response shown to the various tests. From Fig. 15, for example, the Kreis test can be seen to lag behind the peroxide-value much more noticeably in the mixed esters than in the pure oleate. Butter-fat has been observed to give a Kreis reaction nearly 20 times as strong as lard when both fats had absorbed equal amounts of oxygen at 95° C.[139] Response to the Kreis test increases relatively more rapidly than the peroxide-value when the temperature is increased or the fat exposed to sunlight[191].

It would seem, from the data which have now been presented that response to the Kreis test, and presumably to others of the chemical tests which measure secondary products of oxidation, is influenced by the nature of the fat, temperature and light, in much the same way as is the production of a rancid odour and flavour. It might be expected therefore that such tests would show a closer relation to rancidity than estimation either of absorption of oxygen, or of fat peroxide. In varying degree they probably do, but *since no one test measures exclusively that particular mixture of secondary products of oxidation responsible for rancidity—itself not necessarily the same in all cases—it is obviously not possible to define limits for any test above which all fats are rancid and below which all are sweet. Neither can this be done, even for samples of the same fat, if they have been subjected to very different conditions of storage.*

On theoretical grounds, the bisulphite method, which measures both volatile aldehydes and the glyceride-aldehyde residues left when they are split off, and the Schibsted test, which determines the glyceride-aldehydes only, should provide the best measure of rancidity. Further data are required before any pronouncement as to the value of these tests for the direct measurement of the degree of rancidity can be made.

Uses of the Chemical Tests

In the meantime, existing chemical methods can usefully be employed whenever it is desired to compare a number of samples of the same variety of fat, which have been subjected only to fairly similar conditions of storage. In the majority of cases the necessary sensitivity, ease of application and degree of reproducibility, will best be provided by estimation of peroxide. Others of the tests

available may be more suitable in particular cases, e.g., Fahrion's method for soaps, and are generally useful in confirmation. The chemical tests selected should be calibrated against odour and flavour for the particular set of conditions under which they are to be employed.

The more sensitive of these methods will detect in most fats oxidative changes too small in themselves to produce spoilage, but of the greatest importance in determining subsequent keeping properties. It may thus be possible to gauge roughly how far in its induction-period a sample has proceeded, and thence how much longer it is likely to remain good. The use of the chemical tests for determination on the fresh fat of relative potential keeping properties, or relative susceptibility to future deterioration, is of sufficient importance to warrant treatment in a separate section (page 124).

Development of Rancidity in a Limited Supply of Oxygen

In considering the relationship between the chemical tests and rancidity, free access of oxygen has been assumed. Under these conditions rancid (oxidised) fats always give the reactions described above as characteristic of rancidity. The case is different when the total quantity of oxygen available is less than that which can be used by the fat.

In a limited supply of oxygen, the formation of peroxide will gradually fall off as the free oxygen is consumed. Decomposition, however, will still continue, and in a period depending on the temperature and on the stability of the peroxides, they will all have disappeared. It will thus be possible to obtain a fat which contains secondary products of oxidation and may be rancid, but which gives little or no reaction for peroxides. Kilgore[166] quotes figures for cottonseed-oil, sealed up in glass containers under a small volume of air. Peroxide-values after 0, 45, 65, 105 and 125 days' exposure to daylight at room temperature, were 2·3, 6·2, 7·0 and 2·1 ml. 0·002 N. thiosulphate per g., the flavour of the oil at the end of storage being rancid. Similar oil, stirred with exposure to the air, increased steadily in peroxide-content and was not condemned as rancid until after 125 days, when the peroxide had reached 80 ml. per g. Obviously an oil still sweet to the taste but containing an appreciable quantity of peroxide may, in the same way, develop rancidity on storage or more particularly on heating, even in the absence of oxygen.

Formation of the substance responsible for the Kreis test (epihydrin aldehyde) will also proceed in a fat stored in a limited supply of oxygen until most of the latter and the fat peroxide have been used up. Decomposition by secondary reactions may then result in a fall, and possibly in the final disappearance of the Kreis test. In Fig. 17 are plotted the Kreis values recorded for an edible fat stored either with access to the air (friction-top can), or hermetically sealed under small head-spaces filled with various gases.

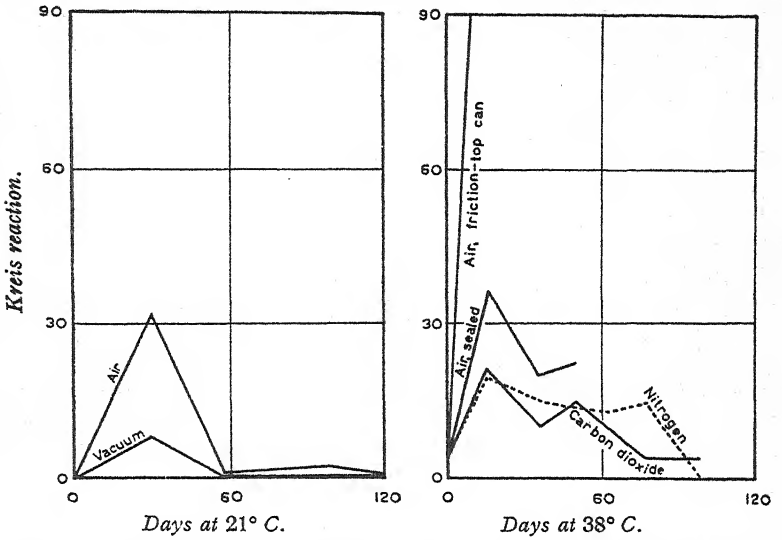


FIG. 17.—*Kreis values for an edible fat, hermetically sealed under various gases.* (Pool[234])

Obviously the peroxide and Kreis tests, at least, must be used with caution in the case of materials which have been exposed to a very restricted supply of air. That the fats can still taste rancid after storage in the absence of air, seems to indicate that the volatile aldehydes and acids mainly responsible for odour and flavour are stable under these conditions. It would certainly be of interest to carry out experiments similar to those described above, but using either the estimation of aldehyde (page 105) or Schibsted's test (page 103) as a measure of rancidity.

Effect of Surface Exposed

In the development of oxidative rancidity, a gas, oxygen, reacts with a liquid or a solid fat.* Except at the surface, where the reaction can take place directly, oxidation must be preceded by solution of oxygen, followed by convection or diffusion into the interior. The area of surface exposed per gram will thus have a pronounced effect on the rate of oxidation of the sample considered as a whole[188].

Oxidation is usually preceded by an induction-period, during which the rate of change is very slow. Peroxide then accumulates rapidly in the fat, which becomes able to accelerate the oxidation of fresh fat to which it is added. Thin films spread on the walls of containers, wrappers, etc., will oxidise much more rapidly than the

* The majority of solid fats contain glycerides which are liquid, or semi-liquid, at room temperature.

main bulk of the material, whose keeping properties may then be adversely affected by contact with the rapidly oxidising films, or its flavour impaired by absorption of volatile products of oxidation. As a case in point may be mentioned the deterioration of baked goods of high shortening content packed in cardboard cartons. Rancidity has been found to develop much more rapidly in the fat-impregnated cardboard separators than in the contents of the carton[241].

Since the diffusion of oxygen into the interior is very slow, the superficial layers of a mass of solid fat oxidise at a much greater rate than the underlying material. For storage it is therefore advisable to keep the exposed surface through which oxygen can diffuse as small as possible. In testing for rancidity in solid fats, a thin superficial layer of standard thickness should be examined. Liquid oils, on the other hand, must necessarily be mixed before testing. The figures thus obtained are useful for comparative purposes, but have no absolute significance.

Effect of Pressure of Oxygen

The effect of low pressures of oxygen on the rate of oxidation of fats is of particular interest, because of the present tendency towards the storage of fat-containing foodstuffs of various kinds, in hermetically sealed containers under either a vacuum or an inert gas, with the object of preventing oxidative rancidity. Examples are dried milk and infant-foods, salad-oils and dressings, cod-liver oil, shelled nuts, sweet corn and coffee. In some few cases the removal of oxygen can be made almost absolute, but in many others the nature of the product is such that dissolved or entrapped air cannot be displaced completely without damage.

Unfortunately, no work on the effect of the pressure of oxygen on the rate of oxidation of pure esters or of fats has yet been published. Theoretically it is quite possible, in an oxidation taking place in the presence of accelerators or inhibitors, for the rate to be almost independent of the concentration of oxygen over a wide range. The rate of oxidation of a dilute solution of ascorbic acid (vitamin C), for example, by dissolved oxygen in the presence of traces of copper is found to be hardly reduced by " deoxygenating " the water by boiling, or increased by saturating the water with oxygen[162].

The amount of oxygen required to bring a fat, particularly one containing a relatively low proportion of acids less saturated than oleic, to the end of its induction-period,* is very small. It is thus possible that reduction in the pressure of oxygen over a considerable range may produce much less than a proportionate decrease in the

* The rate of oxidation during the induction-period is controlled by the natural antioxidant present.

rate of oxidation during the induction-period. In this case a sample stored under a reduced pressure of oxygen might become rancid, when subsequently exposed to the air, almost as rapidly as one which had been under air for the whole period.

It has been observed, for example, that at 0° C. storage in an atmosphere containing only 4 per cent. of the normal amount of oxygen (the rest being carbon dioxide) only increased the induction-period of the fat of chickens' skin to about twice its length in air[187]. Films of lard, stored at 20° C. in salt solutions under air, and air diluted with an equal volume of nitrogen, showed identical induction-periods, but the rate of subsequent oxidation was greater in air[189]. In further experiments[198] acid-extracted, fat-free filter-papers were impregnated with lard and stored at 30° C. under mixtures of oxygen and nitrogen. The majority of the samples in the mixtures containing 100, 21, 8, 3 and 2 per cent. of oxygen exceeded 10 ml. per gram after from 30 to 60 days, but the results were too erratic to distinguish between the various concentrations. Samples in the mixture containing 1 per cent. of oxygen and in commercially pure nitrogen had not exceeded 10 ml. after 60 days. When films of lard were spread on glass slides and stored at 20° C., the samples in pure oxygen exceeded 10 ml. per gram in 80 days, and those in mixtures containing 21, 8, 3, 2 and 1 per cent. erratically in periods between 80 and 120 days. These data all refer to slow oxidation within the induction-period. The rate of subsequent oxidation would presumably be much more sensitive to the pressure of oxygen.

Storage in Inert Gases

Most of the statements by early investigators to the effect that sweet fats become rancid, though stored in inert gases or *in vacuo* can be attributed to the presence in the materials used either of dissolved oxygen, or of pre-formed peroxide. The case of carbon dioxide, however, appears to be exceptional.

Ritsert in 1890 found that lard became rancid in carbon dioxide, while control samples under nitrogen or hydrogen did not. Later workers have also come to the conclusion that carbon dioxide does not behave as an inert gas towards fats[82]; in fact, dried milk has been said to become rancid more rapidly in carbon dioxide than in air[144]. Recently it has been stated that crackers and rice-bran develop rancidity as quickly in carbon dioxide as in air[298, 46].

On the other hand, condensed milk contaminated with copper has been found to develop tallowiness in oxygen and in air, but *not* in carbon dioxide, and the fat of bacon stored in pure carbon dioxide undoubtedly remains in good condition long after control samples stored in air have become rancid (page 217). It is perhaps noteworthy in this latter case that the muscle of the meat would tend to absorb any traces of oxygen remaining in the gas.

Owing to the appreciable solubility of carbon dioxide in fat, samples immediately after removal from storage in high concentra-

tions of this gas possess a sharp taste which disappears on standing in air.

Further evidence from experiments on the storage of peroxide-free fats in the pure gas is necessary before it can be accepted that rancidity can be produced by the action of carbon dioxide.

SECTION 4.—MEASUREMENT OF THE SUSCEPTIBILITY OF FATS AND OILS TO OXIDATION

Since the beginnings of the chemical study of rancidity, methods have been sought which would permit of rapid measurement of the potential resistance or susceptibility of a fresh fat to the oxidative changes which produce rancidity. Such a method has many uses. By its aid suitable fats can be selected for particular purposes; effects on keeping properties of variation in composition, in method of preparation or in handling can be evaluated; incipient oxidation or contamination during processing can be traced; and substances which might be of value for the stabilisation of fats can be tested.

Basically all of the methods which have been employed for determination of susceptibility are similar in principle. Oxidation is accelerated under carefully controlled conditions, while still preserving as far as possible the original relations between the various samples, until the time necessary for the development of spoilage has been reduced from months or weeks, to days or hours. Increase in the rate of oxidation is usually brought about by heating at some constant temperature between 40° and 100° C. in an atmosphere of air or oxygen. In a few cases exposure to light or catalysis by traces of metallic salts has been employed, often supplemented by heat. The progress of the accelerated oxidation is then followed by smell and taste, by direct measurement of the weight or volume of oxygen absorbed, by change in some physical characteristic of the fat, or by chemical estimation of the products of the reaction.

For the determination of the true rate of the reaction between gaseous air or oxygen and a liquid fat, it is essential that the two phases be constantly in equilibrium. This can be attained by keeping the liquid phase always saturated with the gas by stirring or shaking, by bubbling the gas through the liquid, or by dispersion of the oil in the form of a very thin film. A few of the methods described below are empirical in the sense that the rate of oxidation measured depends for reproducibility on exact duplication of the ratio of the surface exposed to the weight, since the samples used do not oxidise uniformly throughout. Nevertheless, results useful for comparative purposes can often be obtained most readily in this way.

A few precautions common to all methods of measuring susceptibility to oxidation must be observed in order to obtain reproducible results. Traces of oxidised fat markedly accelerate the oxidation of fresh samples. Glass apparatus, after cleaning in the ordinary way, should therefore be boiled in a solution of caustic soda, rinsed with chromic acid and finally washed with distilled water. In all

heat-accelerated methods fine control of temperature is essential, since a variation of 1° C. will introduce an error of the order of 10 per cent. into the result. Contamination with metals, and exposure to strong daylight prior to or during the estimation, should be avoided.

Tests by Incubation

The simplest kind of determination of susceptibility, and one that has been very widely used, consists in heating a definite amount of the oil or fat, contained in a glass vessel of fixed dimensions, in a hot-air oven at some suitable temperature, e.g. 100 g. fat at 60° C., and examining at intervals for rancid odour and flavour. Fat-containing foodstuffs can sometimes be tested directly by this method. Disadvantages are the large personal error involved in the organoleptic detection of rancidity, and the length of time necessary to obtain results. In the case of the Schaal modification of this test the time required for common fats has been quoted as from two to more than 40 days[115, 234].

The first objection may be overcome by use of the peroxide-value as a supplement to observations of odour and flavour, and the second in some degree by the use of a higher temperature and a smaller volume of fat. The curves in Fig. 3 were obtained by incubating the fat (10 ml.) in flat-bottomed crystallising dishes (5 cm. diameter) in an electric oven at 60°C.* Six dishes of each sample were used, and were removed one by one for the estimation of peroxide-value and flavour. This simple method is capable of giving very useful results.

Effect of Volatile Products of Oxidation

Objection has been made to the method of incubation on the grounds that volatile products from less stable fats might accelerate the oxidation of adjacent samples. Greenbank and Holm[109, 105] found that when air was led in series through three similar vessels containing butter-fat at 100°C., rancidity developed first in No. 3 (six hours) and last in No. 1 (16 hours), owing to an accelerating effect of the volatile products of oxidation. More recent work by Roschen and Newton[253] does not agree. In this case nitrogen was passed through a sample of rancid lard (peroxide-value, 52 ml. per g.) at 100°C. for four hours, and the volatile products carried over into fresh lard held at a temperature just sufficiently high to keep it melted. The stability of the fresh fat towards oxidation was found to be completely unaffected by this treatment. Heptaldehyde added at a concentration of 0·1 per cent. to fresh lard also failed to produce any effect on susceptibility, whether measured at 70°C. or at room temperature. The conclusion reached was that the materials volatile at 100°C., which include the products responsible for rancid odour and taste, are inactive as catalysts for the oxidation.

It is possible that the discrepancy between the two sets of results is due to the use of air in one case and of nitrogen in the

* 3 g. of fat and 50 ml. pyrex beakers are now used.

other. Traces of volatile peroxides (H_2O_2) or peracids, which are powerful pro-oxidants, might be carried over by the air-stream from the actively oxidising fat. On the other hand, such substances would rapidly disappear from a fat heated under nitrogen. The influence of volatile products on the rate of oxidation cannot therefore be considered as definitely decided, but even assuming an accelerating action, it is doubtful whether any serious interference would be set up between small samples incubated together in a large, well-ventilated oven. The fats should, however, be removed as soon as definitely rancid to the taste, and the oven aerated between determinations.

Measurement of Increase in Weight

One of the oldest methods for the measurement of susceptibility is that involving the determination of the change in weight of thin layers of the oil exposed to air under standard conditions. This has frequently been used for following the oxidation of drying, and sometimes of edible oils,[289, 70] but is not sufficiently sensitive for the investigation of the earlier stages of oxidation in the more saturated fats. In this method the increase measured is actually the difference between the amount of oxygen absorbed and the volatile products lost, so that a *loss* in weight is often recorded during the induction-period, in spite of the fact that the fat is absorbing oxygen. A further error may be introduced by hygroscopic products of oxidation taking up moisture from the air[34]. This method is now rarely used.

Measurement of Absorption of Oxygen

Genthe[98] in 1906 made observations on the uptake of oxygen by linseed-oil, dispersed on filter-paper in a closed system at various temperatures, by means of manometric readings. A similar technique has since been used by others[52, 250]. In more recent practice the oil has been heated in oxygen (a) at 95°C. with stirring[108] (Fig. 33), (b) at 100°C. with shaking[135], or (c) at 70°C. without agitation[110]. Triebold, Webb and Rudy[299] have compared the results obtained by controlled oxidation of lards at 95°, 90° and 70°C. without stirring, and at 95°C. with stirring, and found that in general all the methods ranked the samples in approximately the same relative order of keeping properties.

Methods depending on the absorption of oxygen have been applied to the testing of stability in salad-oils[245], and to studies on the stabilization of fats by antioxidants[208]. Moureau and Dufraisse[216] used such a method in their classical researches on the effects of accelerators and inhibitors on autoxidative reactions.

In the simplest form of the method the oil is placed in a flask immersed in a thermostat and connected by a ground-in stopper, preferably with a mercury-seal, to a manometer. The apparatus is then filled with oxygen,* and the absorption followed by observation

* Air may be used.

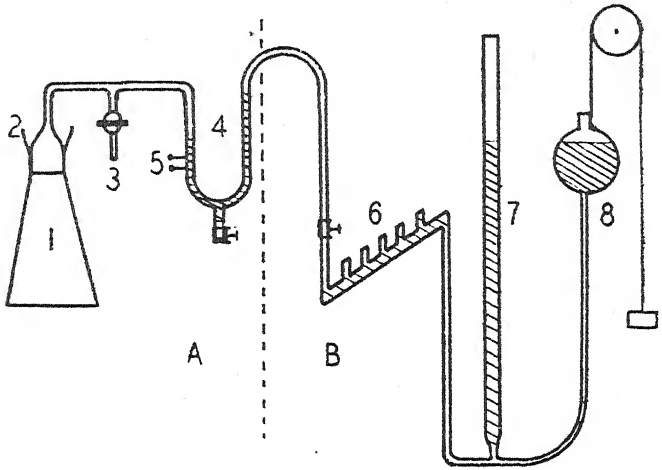


FIG. 18.—*Apparatus for the measurement of the absorption of oxygen by fats by the "static" method* (French, Olcott and Mattill [92]).

(A). System for determination of the induction-period. (1) 250 ml. flask with ground-in connection, immersed in thermostat. (2) Lip for mercury seal. (3) Side arm for introducing oxygen. (4) Manometer. (5) Electrical contacts for indicating end of induction-period when mercury is used in manometer. (B). System for measurement of absorption of oxygen at intervals. (6) Common connections for battery of flasks. (7) 50 ml. burette. (8) Counterpoised levelling bottle. The water is shaded in the glass, unshaded in the rubber parts of the manometric system.

of the fall in pressure. The manometer may operate a continuous recording device[234], or alternatively close a circuit and ring a bell or mark a clock-driven drum, when the pressure within the system has fallen by some pre-determined amount. Mattill[206, 92], who describes this procedure in detail (Fig. 18), has shown that for sufficiently small amounts of fat (*e.g.*, 5g. in 250 ml. flask at 70° C.), the rate of oxidation is independent of the quantity used, indicating that saturation with oxygen is being obtained. The more complicated apparatus in which the fat is stirred at a high speed by a gas-tight stirrer[108, 31] therefore seems unnecessary.

The " static " form of the method can be used in some cases to measure the susceptibility of fat-containing materials as well as of pure fats. Holm, Greenbank and Deysher[143] in this manner investigated the effect of variations in the method of preparation, and in the conditions of storage, on the stability of dried-milk powders. More recently it has been shown that a much better indication of the keeping properties of crackers is given by determinations of susceptibility on the biscuits themselves, rather than on the shortenings used in their manufacture[299]. This is probably owing to the influence of the natural antioxidant of the oil of the flour, and to the effect of mixing and baking on the stability of the shortening. Samples of lard, ranging in initial peroxide-value from 1·6 to 41·5 ml. 0·002 N. thiosulphate per gram, and in induction-period from zero to three hours, produced crackers of approximately the

same induction period (8–9·5 hours). Examination of the fats recovered from the crackers showed that most of the peroxide originally present in the shortenings had been destroyed during baking. Other data[24] gave induction-periods (in hours) as follows: lard shortenings, 5–10; crackers, 2–6: oleo-oil shortenings, 5–8; crackers, 6–15: hydrogenated shortenings, 30–120; crackers, 15–40. Mixtures of two shortenings of different stability gave results intermediate between those of the components[23].

The rate of absorption of oxygen in autoxidative reactions is influenced to some extent by changes in pressure[210]. The manometric method is therefore best suited to measurement of the induction-period, during which changes in pressure are slight. The time required for 10 ml. of the oil to absorb 3 ml. of oxygen is sometimes taken as an "oxygen-absorption value," by means of which the susceptibilities of different fats are compared. It is probably sounder practice to use a different standard for each type of fat, preferably making comparison at the point at which rancidity first appears under the conditions of the test, or at the end of the induction-period.

The usefulness of this method for indicating susceptibility to oxidative rancidity has been confirmed by many investigators. It is, however, less sensitive than the peroxide methods for measurements of changes *during* the induction-period. Furthermore, the fall in the pressure of oxygen and the accumulation of volatile products of decomposition tend to produce an apparent decrease in the rate of absorption in the later stages of oxidation[135]. This can be overcome by working at constant pressure and absorbing volatile products[210], but the technique then becomes too complicated for routine determination of susceptibility.

The rate of absorption of oxygen by oils and by unsaturated fatty acids under physiological conditions may be measured by means of the Barcroft or Warburg technique[160].

Methods depending on the Schiff and Issoglio Tests

Objection has been made to the oxygen-absorption method on the grounds that the volume of oxygen taken up bears no constant relationship to flavour, which is due to a mixture of volatile secondary products of oxidation, predominantly aldehydes. In a method[14] devised to overcome this difficulty air is passed at a definite rate over the oil (3 g.) dispersed on filter-paper in a jacketed tube at 92°C. and then through Schiff's solution, the time for the appearance of a pink colour being taken as a measure of susceptibility. Results have been found to agree closely with tests by incubation in which odour and taste were used as criteria of rancidity[310].

Grettie and Newton[115, 116] criticised this procedure on the grounds that the Schiff reagent in which the volatile products are condensed is somewhat unstable, and that it is difficult to measure the colour produced. They therefore substitute acid potassium

permanganate solution for the Schiff reagent, and estimate the volatile oxidisable substances as in the Issoglio test.

The fat (2 g.) is dispersed on filter-paper and placed in a glass tube surrounded by a boiling water jacket. Air is drawn through a wash-bottle, over the fat, through 10 ml. of 0·01 N. permanganate immersed in a water-bath at 25°–30°C., and through a capillary tube which, with the aid of a device for regulating the pressure, adjusts the rate of flow to 1 ml. per second. Several determinations can be run in parallel. The tubes of permanganate are replaced and titrated at regular 20 minute intervals until the evolution of volatile products is sufficiently rapid to reduce 1 ml. of 0·01 N. permanganate in one 20 minute period. For samples of exceptional stability, a period of one hour may be used. These figures were selected arbitrarily, as corresponding roughly to the point at which the odour of rancidity was observed to develop.

This method gives useful results, but requires more apparatus and attention than the majority of the others. The simplification that has been achieved in the peroxide methods by dispensing with an accurately controlled air-flow, cannot safely be applied here, since the current of air, in addition to oxidising the fat, is also responsible for carrying over the volatile products. It is therefore less widely used than the peroxide, incubation, and static absorption methods.

Methods using the Reduction of Methylene Blue

Two methods for determination of susceptibility involving the use of methylene blue have been described.

In that due to Davies[60], 1 g. of the oil, together with 1 ml. of a 0·25 per cent. solution of methylene blue, is emulsified in 10 ml. of diluted, separated milk, and incubated at 37° C. until the blue colour has been bleached by the " reductase " of the organisms present in the milk. The contents of the tube are then well shaken with air for 15 seconds and allowed to stand for two minutes. The depth of the blue colour produced is considered to be an indication of the capacity to oxidise of the fat under examination.

This test has been applied to the detection of metallic contamination in butters, the colour being restored in proportion to the amount of copper or iron present.

The second method is based on the observation that when an oil or fat containing methylene blue is exposed to light, the unsaturated constituents of the fat are oxidised and the dye reduced[1]. It has been suggested that the dye acts as an acceptor of hydrogen in the reaction in which unsaturated fat is oxidised[316]. On the other hand, it may be that oxidation of the fat, accelerated by light, reduces the concentration of dissolved oxygen until the oxidation-reduction potential corresponding to total reduction of the dye is reached[61]. Dyes of the eosin-erythrosin type behave similarly[146]. Greenbank and Holm[112] have made use of the methylene-blue reaction in a photochemical method for the determination of the relative susceptibilities of fats and oils to oxidation.

A mixture of the dry fat (20 ml.) with a 0·025 per cent. alcoholic solution of methylene blue (1–2 ml.) is placed between a 100 watt tungsten lamp and a photo-electric cell. As the dye bleaches, a gradually increasing amount

of light is transmitted by the oil, until at a pre-arranged point the amplified current from the cell actuates a relay and operates a buzzer or other signalling device.

Results obtained in this way showed excellent agreement with those from the determination of induction-periods by the method of measuring the absorption of oxygen (Table 33).

TABLE 33.—*Susceptibility of milk-fats as determined by the methods of absorption of oxygen and reduction of methylene blue.*
(Greenbank and Holm[112])

Age of sample (weeks)	Induction-period		Bleaching time	
	mins.	%	mins.	%
0	183	100	32	100
2	150	82	26·3	81·5
6	122	66·7	21·6	66·0
8	100	53·8	16·3	53·6

This procedure has been simplified by Royce,[254, 255] who substitutes visual for photoelectric control, and irradiates with a 100 watt lamp at a distance of 15 cm. in a thermostatically controlled oven at 70°C. Six tubes, each containing 25 ml. of oil and 1 ml. of a 0·025 per cent. alcoholic solution of methylene blue, are irradiated simultaneously.

Methods depending on Estimation of Peroxide

Probably the most satisfactory means at present available for following the earlier stages of atmospheric oxidation in edible fats and oils is by the determination of peroxide oxygen (page 106). This method within the last few years has become widely used in work on susceptibility. The manner in which the fat is brought into contact with oxygen and the means employed to accelerate the reaction, vary considerably in the different techniques.

Acceleration by Light

Lea[193, 191] exposed the fat in small flat-bottomed glass dishes at a constant temperature of 25°C. to the light of a 100 watt lamp at a distance of three feet, four dishes of each of four samples being irradiated by one lamp. In this way it was shown that perfectly fresh fats from different individual animals of the same species may show large (4:1) differences in susceptibility to oxidation. The determination is slow, but can be shortened by the use of an incubator at a higher temperature.

In a recent method the time of exposure has been further shortened by irradiation with ultra-violet light (intensity standardised by

photoelectric cell), oxidation being followed by means of the Issoglio, Kreis and peroxide tests[177].

Acceleration by Heat

A comparison of the susceptibilities of fats to oxidation can be obtained by heating the samples, dispersed on small fat-free filter-papers, in an oven at 70° or 100°C.[188] Several papers prepared from each sample are suspended by loops of cotton from a glass rod carried on a rack in the centre of the oven. Peroxide oxygen is estimated at intervals, a paper carrying the fat being transferred directly to the test-tube used for the determination. In using this method simultaneous determinations on samples of very different keeping properties have been avoided. It is doubtful, however, whether this precaution is necessary, so long as any remaining papers are removed from the oven as soon as the fat has reached the rancid point (page 126). A temperature lower than 100°C. is preferable for readily oxidisable fats, in order to reduce the effect of fluctuations of temperature caused by opening the oven. The curves shown in Fig. 19 were obtained by this method.

The Method of Aeration

Wheeler[311] measured susceptibility by bubbling air at a constant rate through the oil at 100°C., determining the peroxide-value at intervals. A modified form of this technique due to King, Roschen and Irwin[168], and usually referred to as the "Swift Stability Test," has been adopted in a number of American laboratories. A method of this kind combines the accuracy and reproducibility of the estimation of peroxide with speed of operation and the advantage of measuring susceptibility in terms of the time required to reach the actual point at which rancidity appears.

The tubes are immersed in a bath of mineral oil, maintained at a constant temperature slightly below 100° C., by a boiling-water jacket. Air, washed by passage through permanganate solution, is drawn through the fat (20 ml.) in each of a number of tubes at a rate controlled by a calibrated capillary and constant-pressure device to 2·33 ml. per second. Three tubes are started at intervals of one hour (two, three or five hours in the case of very stable fats). When the first sample has become rancid, as indicated by the detection of a rancid odour in the gas issuing from the tube, all three are removed, cooled by immersion in cold water, and peroxide-values determined on the fat. The number of hours required for the peroxide-value to exceed a figure, found by trial to correspond to the appearance of rancidity (20 ml. 0·002 N. thiosulphate per g. in case of lard), is taken as a measure of stability. If greater accuracy is required, a larger number of tubes may be used and the curve plotted in the region of the point at which rancidity appears.

Table 34 gives an interesting comparison of determinations of susceptibility made independently on four samples of lard by four laboratories using different methods.

All seven methods place the samples in the same order[93]. The amounts of time, labour and apparatus necessary, however, vary widely, and the method selected by a particular laboratory will doubtless depend largely on the relative importance attached to such

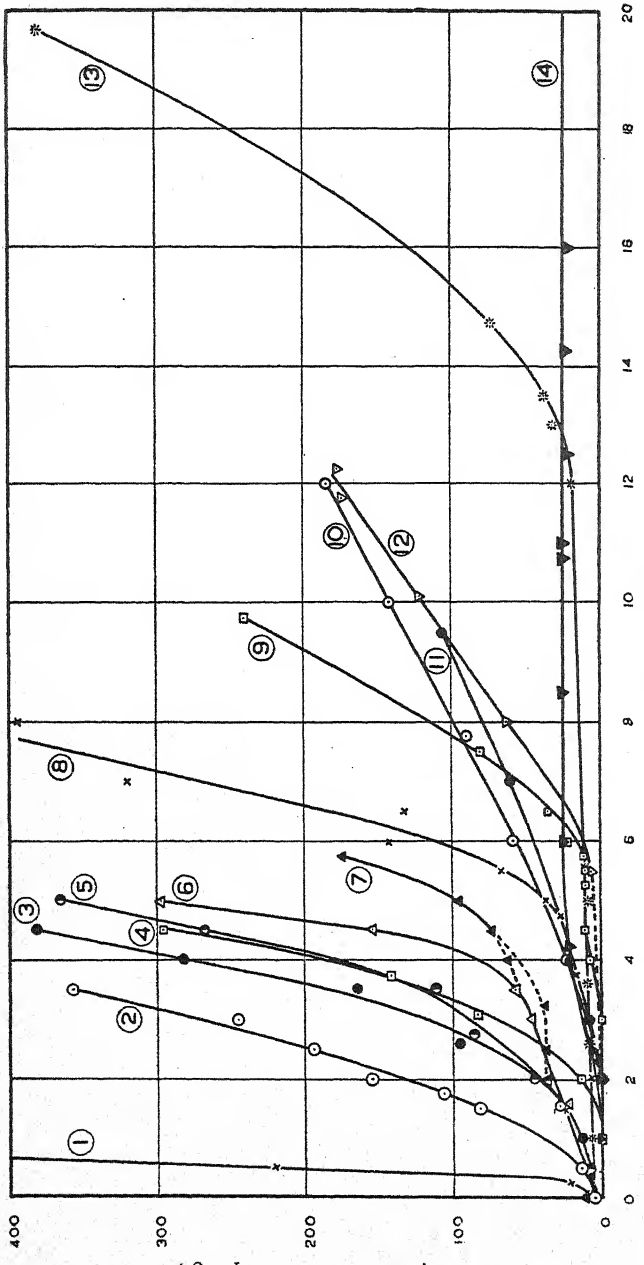


FIG. 19.—*A comparison of the rates of oxidation of various natural oils and fats.* (Lea[188])

1, Cod-liver oil; 2, 3 and 4, lard; 5, olive-oil; 6 and 7, cottonseed-oil; 8, vegetable cooking fat; 9, beef- brisket fat; 10 and 11, beef-kidney fat; 12, butter-fat; 13, castor-oil; 14, egg-oil.

TABLE 34.—*A comparison of several methods for the determination of susceptibility.**

(King, Roschen and Irwin[168])

Sample	Test A (hours)	Test B (hours)	Test C (hours)	Test D (ml. per g.)	Test E (hours)	Test F (days)	Test G (days)
I	2	6·0	7·5	23·5	9·4	3	8
II	7	14·3	17·0	5·0	38	5	18
III	12	21·7	28·3	3·5	52	8	31
IV	20	35·2	35·0	2·75	75	12	39

* TEST A.—"Swift test," aeration at 97·8° C. (Hours to become rancid.)
TESTS B AND C.—Absorption of oxygen at 90° C., with recording manometer. (Hours required for 10 ml. of fat to absorb 3 ml. of oxygen.)
TEST D.—20 g. samples incubated at 70° C. for 3 days and peroxide-values determined. (ml. 0·002 N. thio. per g.)
TEST E.—10 g. samples incubated at 79° C. until rancid. Odour checked by peroxide-values. (Hours to become rancid.)
TEST F.—20 g. samples incubated at 70° C. and examined for odour. (Days to become rancid.)
TEST G.—The Schaal test. 50 g. samples incubated at 60° C. and examined daily for rancid odour. (Days to become rancid.)

factors. The methods which use chemical control are the most sensitive, and have the advantage of yielding numerical results which are independent of the personal factor.

The committee on methods for the determination of stability, set up by the American Oil Chemists' Society, has recently (1935) reported as follows[4]. "The two most useful accelerated tests for judging stability are the oxygen absorption test in its various forms, and the active oxygen or peroxide test as developed by Lea, Taffel and Revis, Kilgore and Wheeler, and by King, Roschen and Irwin. A consideration of these two methods indicated that the peroxide test could be adapted to routine laboratory operation more easily than the oxygen absorption method . . . As far as this committee is aware, the peroxide method for judging the stability of edible oils and fats is being used more and more all the time . . . In conclusion, this committee is of the opinion that in the hands of an experienced operator the active oxygen or peroxide method is not only the best routine accelerated test available, but is also reliable and worthy of the support of this society and of the edible fat industry."

Recent Improvements in the Aeration Methods.—During the last year some useful modifications of the above technique have appeared

In the methods of Wheeler and of King, Roschen and Irwin, the rate at which air passed through the samples was controlled by a separate calibrated flowmeter or capillary for each tube. The object of this somewhat troublesome procedure was to maintain

the oils uniformly saturated with air, and to carry away always the same proportion of volatile products, which were believed to influence the rate of oxidation. Actually any rate of flow above quite a low minimal value will suffice to maintain the oil saturated with air, and recent work has made it doubtful whether volatile products seriously influence the reaction (page 125). It has been observed on several occasions that large variations in the rate of flow of oxygen through oils kept at 100° C.[320], or irradiated with ultra-violet light[147], have not appreciably influenced the rate of oxidation. The effect of different rates of air-flow and different types of jet on the values obtained in the test for stability has now been investigated[167]. Except for erratic fluctuations over a range of about 5 per cent., no detectable change in the rate of oxidation was found for a variation in the flow of air from 2·5 to 10 litres per hour, or in the size of the bubble, from the cloud of small bubbles produced by a sintered glass jet to the few large bubbles produced by a bell or funnel-end. Thus a simple aeration-tube, made from ordinary glass tubing and delivering a relatively small number of bubbles per minute, e.g., just above counting rate, gives an adequate supply of oxygen and efficient stirring, and produces no appreciable reduction in the accuracy of the method.

A cheap and easily constructed apparatus which omits the oil-bath has been described[94]. The glass tubes containing the oil fit directly into lengths of 1-in. copper pipe, closed at the lower end and soldered into the top of a rust-resisting closed container in which water is kept boiling by means of an electric immersion-heater and retained by a reflux condenser. Detection of the rancid odour is facilitated-by the collection in a small flask of a sample of air from the outlet tube.

Since at 100° C. four days might be required for very stable fats of the all-hydrogenated type to become rancid, a modification was proposed in which the boiling water is replaced by a thermostatically controlled, electrically heated oil-bath at 119° C. A reduction of about three times in the period required for the test is thus obtained.

A chemical method has been suggested for the determination of the approximate end of the induction-period as an alternative to the detection of a rancid odour as usually employed. The air-outlet tube is immersed to a mark 10 cm. from its end in a test-tube of water containing 1 ml. of 0·01 N. sodium hydroxide and coloured with methyl orange. The three samples are removed when the first tube has turned deep red, but before the last has begun to change. If hydrolytic rancidity is present in a fat containing volatile fatty acids (e.g., butter), the red colour will develop within a few minutes of starting. In such a case it is necessary to replace the test solution at the end of the first hour, after the acids originally present have been driven over. This method is stated to be of advantage in cases where the odour of the fat may mask that due to rancidity[277].

The Use of Light and Metals

Even when aeration at 100° C. is used, the length of time necessary for the determination of susceptibility on stable fats considerably exceeds one working day, and several attempts have been made to reduce the time required to within this limit. Irradiation by a 200 watt lamp[256], and introduction of a metallic catalyst (copper or manganese),[256,169] have both been used to shorten the test.

In the case of the lamp it is desirable to check the constancy of the light emitted by means of a photoelectric meter, since fluctuation in the voltage of the supply and ageing of the filament may appreciably affect the intensity of the light. Experience with metallic catalysts is, at present, somewhat limited, and it is possible that they may prove difficult to control. In any case care is necessary in interpreting results obtained in this manner, since some substances which appear to be powerful antioxidants, as judged by their efficiency in inhibiting the accelerated reaction, are useless as inhibitors in the absence of the metal (e.g., cyanide, page 178).

Physical Methods

Changes in physical properties which occur when a fat oxidises have found but little use in the measurement of susceptibility. The refractive index and viscosity both rise as oxidation proceeds, but the increases during the early stages of the reaction are small. Recently it has been claimed[28] that direct measurement of the surface-tension between a 1 per cent. solution of the fat in benzene and 0·002 N. sodium carbonate, by means of the du Noüy tensiometer or by the Dubrisay drop method, furnishes a rapid and accurate method of investigating the deterioration of fats by autoxidation. No further data are available on the use of this method.

Of interest from the point of view of research is the spectroscopic method of Trillat[300] in which the X-ray spectra of unsaturated acids, spread in thin films on lead or glass plates, are examined. Spectra observed have been attributed to the acid or its lead salt, the peroxide and (in case of the more highly unsaturated acids) the completely polymerised material.

Reference has already been made to the method in which changes in force/area and interface potential/area relations are utilised for following oxidative changes in monomolecular films of unsaturated acids, esters or glycerides (page 92).

SECTION 5.—THE MECHANISM OF AUTOXIDATION

Before proceeding to a discussion of the effect of accelerators and inhibitors on the oxidation of fats, it will be necessary to consider briefly a few of the more important characteristics of autoxidative reactions in general. As already pointed out (page 79), many

chemical substances other than unsaturated fatty acids and glycerides, oxidise spontaneously when exposed to oxygen. Certain criteria characterise reactions of this type.

(a) They appear to be "autocatalytic," i.e., oxidation proceeds at a rate which increases up to a limiting value, and ultimately falls off. The curve obtained from the oxidation is thus of a characteristic S-shape.

(b) They are extremely susceptible to the presence of traces of foreign substances, which may either accelerate or retard the reaction.

(c) They induce the oxidation of other substances which are normally relatively stable towards free oxygen.

(d) They induce polymerization in various autoxidisable substances.

The Induction-period

The reaction of an autoxidisable substance with oxygen is usually characterised by a phase of very slow change which precedes rapid oxidation. The explanation originally advanced to account for this induction-period[98], and one that until recently has been generally accepted, was that some product of the reaction most probably the peroxide, functions as a positive catalyst in promoting further change. This substance was considered to accumulate in the fat until present in sufficient quantity to bring about the rapid oxidation. Much experimental data, some of which has already been mentioned, appeared to support this view. Certainly, the addition of small quantities of an oxidised sample to a fresh fat reduces the induction-period of the latter[307], and the formation of traces of products of oxidation *in situ* by exposure to light increases the rate of subsequent oxidation. An alternative explanation of these facts is, however, possible.

Recent work has shown in several cases that the pronounced induction-period observed with ordinary specimens of autoxidisable substances becomes progressively shorter as the substance is purified. In this way, for example, the induction-period of cyclohexane has been reduced to a very small value[280], and that of benzaldehyde (Fig. 28) completely eliminated[243,2]. In the case of fats, the induction-period of the glycerides resynthesised from the distilled acids of a natural oil, has been found to be only a small fraction of that of the original oil[135]. The induction-period (at 80° C.) of methyl oleate prepared from olive-oil has similarly been reduced from 14 hours for the oil to one hour for the ester[123]. Yamaguchi[320] claims that pure triolein oxidises without any induction-period, and it may be that even the small values observed in the other cases are due to traces of inhibitors still present in the final specimens.

Thus, there is now direct evidence that the induction-periods shown by many autoxidisable compounds are due, in the major part at least, to the presence of traces of foreign substances. According to this theory the pro-oxidant effect of the products of the oxidation of fat (peroxides) will be largely an indirect one, resulting from their destructive action on these antioxidants.

Theories of the Action of Inhibitors

Moureau and Dufraisse[216, 217] put forward two theories to account for the results of their extensive researches on the inhibiting effects of traces of foreign substances on autoxidative reactions. According to these, the antioxidant (A) reduces the active peroxide formed from the autoxidising substance (F), being itself oxidised in the process.

$$F + O_2 \longrightarrow FO_2 \qquad FO_2 + A \longrightarrow AO_2 + F$$

Alternatively, the antioxidant is considered to oxidise, either directly or by interaction with FO_2 as above, and the peroxides thus formed mutually to reduce one another after the manner of hydrogen peroxide and ozone.

$$FO_2 + AO_2 \longrightarrow F + A + 2O_2$$
$$\text{or } FO_2 + A \longrightarrow FO + AO \longrightarrow F + A + O_2$$

Recent work has made it clear that these theories cannot adequately account for the experimental facts, unless supplemented by the assumption of some kind of chain-mechanism.

According to the chain-theory of Christiansen,[42, 3, 22] an activated molecule of the autoxidisable substance† and a molecule of oxygen unite with the production of a peroxide and the liberation of a considerable amount of energy. The freshly formed peroxide molecule is able to pass on this energy to another (or possibly to more than one) molecule of the substance, thus initiating a reaction-chain (or chains) which may be many thousand molecules long. The actual length of the reaction-chain will depend on the efficiency with which the energy is transferred, and on the presence of foreign substances.

$$F + \text{energy} \longrightarrow F^* \qquad F^* + O_2 \longrightarrow FO_2^*$$
$$FO_2^* + F \longrightarrow FO_2 + F^* \qquad F^* + O_2 \longrightarrow \ldots\ldots\ldots$$

When an activated peroxide molecule comes into contact with a molecule of inhibitor, the latter takes up the energy, and as a result is usually, but not invariably, oxidised itself in subsequent collisions‡ with oxygen. It fails, however, in its turn to activate any further molecules of the autoxidising substance, and thus the reaction-chain is broken.

$$FO_2^* + A \longrightarrow FO_2 + A^* \qquad A^* + O_2 \longrightarrow AO_2$$

If, for example, into a substance whose normal chain-length is 10,000, 0·1 mole per cent. of an inhibitor is introduced, then the probable number of energy-transferring collisions that will take place before the energy is lost by collision with an antioxidant

† It is assumed that the reaction takes place between activated molecules (denoted by *) of the substance and oxygen. The same reasoning holds if it is the oxygen molecules, or both, that are activated.

‡ The mechanism of chain-reactions has been worked out mainly for reactions in gaseous and liquid systems, but presumably energy can be similarly transmitted from molecule to molecule in the solid state, as, for example, in the detonation of solid explosives.

molecule will be 1,000. In other words, the average chain-length will be reduced from 10,000 to 1,000, with the result that the velocity of the reaction is reduced to one-tenth of its original value. This figure depends on the assumption that the efficiencies of transference of energy to reactant and inhibitor are the same. If energy is transferred more efficiently to the inhibitor than to the reactant, the effect on the velocity of the reaction may be much greater.

Obviously, too, according to the chain-theory, acceleration of the autoxidation is possible by any factor capable of activating molecules of reactant, and so of initiating reaction-chains, e.g., radiant energy (heat or light), chemical energy (oxidation of another substance), positive catalysts, etc.

SECTION 6.—FACTORS WHICH INFLUENCE THE RATE OF OXIDATION

Accelerators of the oxidation of fat may conveniently be grouped into several classes, namely, (*a*) radiation, i.e., heat and light, (*b*) oxidising agents, such as peroxides, peracids and ozone, nitrous acid and certain organic nitro-compounds, aromatic aldehydes, etc., (*c*) metallic catalysts, particularly salts of certain of the heavy metals, and (*d*) oxidase systems, i.e., heat-labile organic oxidative catalysts.

The Effect of Temperature

In common with all chemical reactions, the rate of oxidation of a fat exposed to the air is increased by raising the temperature and decreased by reducing it. In the majority of the fats of food the reduction in the rate of deterioration brought about by the conditions of commercial cold storage is sufficient to maintain the product in edible condition for long periods. In some cases, however, the temperatures ordinarily employed are not sufficiently low to preserve fat-containing foodstuffs for the length of time which seasonal production, oversea transport and fluctuating markets render desirable. The cases of fish-oils, rabbit's fat and the fat of bacon, all of which oxidise comparatively rapidly at −10° C., are treated in other sections.

The effect of temperature on the oxidative reaction is of the order usually found for chemical processes. Thus, the rate of accumulation of peroxide during the aeration of oils between 100° and 115° C. is approximately doubled for a rise in temperature of 10° C.[94] This factor may be rather lower than that for the actual rate of formation, owing to a relatively more rapid decomposition of the peroxide at higher temperatures. Calculation from data given by Yamaguchi[320] for the duration of the induction-period (absorption of oxygen) of olive-oil at various temperatures, gives a coefficient of 2·4 between 80° and 100° C. and 2·5 between 100° and 120° C. Lea has obtained a temperature coefficient of 2·6 for the rate of accumulation of

peroxide in thin films of herring-oil at temperatures between 0° and −20° C[196].

The case is different when the fat is exposed to light, or when metallic catalysts are present. Cod-liver oil oxidising slowly at temperatures between −9° and 27° C. under the influence of relatively weak illumination, gave a temperature coefficient of approximately 1·8[191]. On the other hand, the rate of oxidation of

TABLE 35.—*Effect of temperature on the oxidation of linseed-oil in the presence of a metallic catalyst.**

Cobalt (as linoleate)	Minutes to absorb 10% of oxygen at (° C.)			Temperature coefficient	
	40°	50°	100°	40°–50°	50°–100°
—	2,113	757	29	2·8	1·9
0·0003%	847	346	12	2·45	1·9
0·003%	185	84	12	2·2	1·5
0·03%	71	44	12	1·6	1·3

* Calculated from curves given by Rogers and Taylor.[250]

linseed-oil and of linoleic acid under powerful irradiation with ultra-violet light has been found to be practically unaltered by variation in temperature from 0° to 25° C. (temperature coefficient, 1·02–1·04)[147]. Similarly, the presence of a metallic catalyst reduces the effect of temperature on the rate of oxidation (Table 35.)

Obviously, oxidation in the absence of light and of positive catalysts has a normal temperature coefficient, but the importance of temperature becomes progressively less as the intensity of illumination or the content of active metal increases. No determination has yet been made of the effect of temperature on an oxidase-assisted reaction.

The Influence of Light

Ritsert (1890) and other early investigators in this field observed that exposure to light has a marked effect in accelerating the development of rancidity. Subsequent reports showed considerable confusion regarding the part played by light, some asserting that light and oxygen are both essential, while others claimed that light alone, in the absence of oxygen, is capable of producing rancidity[308]. It is now known that sweet fats stored in the complete absence of air, but with exposure to light, may sometimes become rancid through decomposition of peroxides already present in the "fresh" oil.[139, 142] Peroxide-free fats stored under pure nitrogen apparently do not become rancid when exposed to daylight[166]. Even intense irradiation of linseed-oil[147] or of linolenic acid[102] with ultra-violet light in the absence of oxygen fails to produce any appreciable change in the iodine-value, while that of control samples in oxygen

rapidly falls (Fig. 13). It would seem from these results that the action of light consists essentially in accelerating the oxidation of unsaturated constituents of the fat, and that irradiation in the complete absence of oxygen does not produce rancidity.

Exceptions to this generalisation are provided by the recent work of Schmalfuss and collaborators[265, 295], who have shown that many substances, including saturated and unsaturated fatty acids and glycerides, give rise to small quantities of odorous aldehydes and methyl ketones when subjected to prolonged heating or exposed to ultra-violet light. Ketones and acids of lower molecular weight are produced more readily from unsaturated than from saturated acids, and more readily from those containing 12 or fewer carbon atoms than from palmitic and stearic acids. Oxygen and moisture are not essential, but assist the reaction. The free hydroxyl groups of mono- and di-glycerides are oxidised to aldehyde groups by irradiation with ultra-violet light in the presence of oxygen[137]. Methyl laurate and glycerol, irradiated for long periods under similar conditions, have been found to give first the Schiff and then the Kreis tests, indicating some decomposition with the formation of aldehydes[266]. The mechanisms of these reactions are by no means clearly understood.

The possibility of decomposition of the saturated fatty-acid chain should not therefore be overlooked in the consideration of oxidative rancidity. Nevertheless, exposure of the fats of food to ordinary daylight for reasonable periods is harmful mainly through its effect in accelerating autoxidation of the unsaturated glycerides.

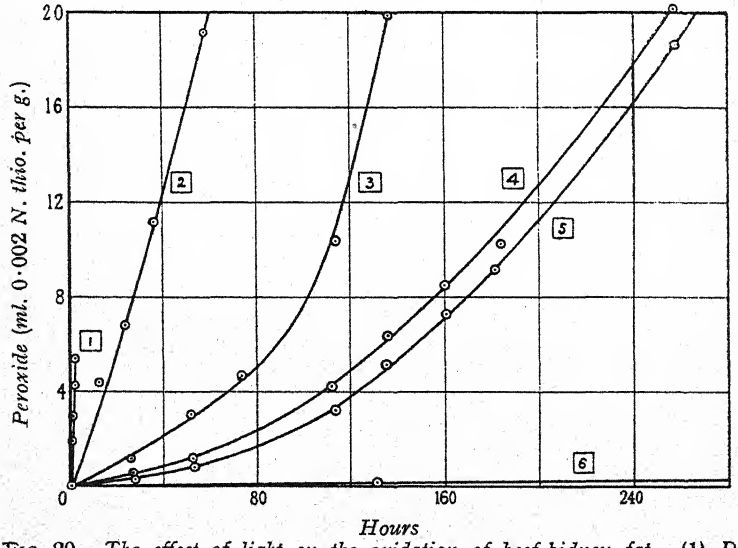


FIG. 20.—*The effect of light on the oxidation of beef-kidney fat.* (1) *Direct sunlight (March).* (2) *Diffused daylight, north window (March).* (3, 4 *and* 5) 2, 5, *and* 6 *feet from a* 100 *watt lamp.* (6) *Darkness.* (Lea[191,192])

Magnitude of the Effect of Light

The degree of acceleration produced by exposure to light varies with the nature of the fat, and appears to be greatest in those samples which are most stable in the absence of light. Under the influence of strong illumination, the pronounced induction-period which usually precedes rapid oxidation becomes very much reduced, and may disappear completely. Fig. 20 illustrates the type of effect observed when a fresh fat, capable of considerable resistance to oxidation,* is exposed to light. The peroxide-values used for following the oxidation do not indicate the full measure of the increased rate of spoilage produced by direct sunlight, since under these conditions rancidity appears at abnormally low contents of peroxide (page 115). Exposure to direct sunlight for five or 10 minutes is sufficient to produce perceptible rancidity in fats such as butter or beef-kidney fat, which contain only small proportions of acids less saturated than oleic.

Comparatively little work has been done on the quantitative relation between intensity of illumination and the acceleration of oxidation produced. Rogers and Taylor[250], investigating the absorption of oxygen by linseed-oil under irradiation by a quartz-mercury lamp, found that the rate of oxidation (negligible in the absence of light), was not directly proportional to the intensity of the irradiation, but that the weaker the light employed, the greater was its efficiency in promoting oxidation. At intensities proportional to 16, 4 and 1, the numbers of molecules reacting per quantum of light-energy absorbed were approximately 0·3, 0·6 and 1·3.

Recently Horio[147] has oxidised linseed-oil and linoleic acid during exposure to ultra-violet light at 0·5° and 25° C., following the course of oxidation by estimation of the peroxide produced. His results show that the velocity of the photochemical reaction is proportional, not to the intensity of the light, but to its square root (Table 36). The results of Rogers and Taylor fit in well with this relation.†

Some early results obtained by Lea[191] for beef-fat oxidising with a pronounced induction-period in weak light, have been shown[213] to agree with simple proportionality to the exciting light, *plus* an abnormally large effect for light of very low intensities. The latter observation is of doubtful reliability, since it is possible that the precautions taken to exclude light from the control samples also reduced the evaporation of moisture, and thus further reduced the rate of oxidation. Morgan's data connecting the increased rate of development of rancidity (which depends rather on the length of the induction-period than on the rate of subsequent oxidation) with the intensity of the light, also indicated that the effect was directly proportional to the exciting light.

* The peroxide-content after storage for 1,200 hours at 25° C. in the absence of light was still below 1 ml. per g.

† After correction for several misprints in Table VI of the original paper.

TABLE 36.—*The relation between intensity of light and formation of peroxide at 25° C.*
(Horio[147])

Substance	Intensity of light (I)	Peroxide formed per hour* (P)	$\frac{P}{I}$	$\frac{P}{\sqrt{I}}$
Linseed-oil ..	6·5	4·68	0·72	1·84
	6·5	4·34	0·67	1·70
	3·5	3·63	1·04	1·94
	1·1	1·84	1·67	1·75
	1·1	1·99	1·81	1·90
			average	1·83
Linoleic acid..	14·0	3·54	0·253	0·945
	6·5	2·35	0·362	0·924
	3·5	1·69	0·483	0·904
	1·7	1·19	0·700	0·915
	1·1	0·96	0·873	0·914
			average	0·921

* ml. 0·1 N. thiosulphate per 5 g.

It may therefore be concluded that, at temperatures sufficiently low for the thermal reaction to be negligible, fats exposed to strong illumination with free access of oxygen, oxidise with little or no induction-period at a rate which varies as the square root of the intensity of the light. When, on the other hand, the thermal reaction is rapid, the effect of light of moderate intensity is practically confined to reducing the length of the induction-period. Thus, irradiation of butter-fat by a quartz-mercury lamp at 100° C. has been found to reduce the induction-period from 17·3 to 5·5 hours, though very little effect was observed on the rate of subsequent absorption of oxygen[31]. Even at ordinary temperatures exposure to weak light is mainly important because of its effect on the induction-period. The rate of oxidation in strong light, however, is very much greater than that of the post-induction period in darkness. The relation between intensity of light and rate of oxidation *within* the induction-period has not been satisfactorily elucidated, but may be one of direct proportionality.

Autocatalytic Nature of the Reaction

The form of the typical curve for autoxidation of a fat shows that the process is autocatalytic, some product of the reaction serving as a positive catalyst* for subsequent oxidation. When a fat is exposed to light, this catalyst (presumably a reactive peroxide) is formed, and subsequent removal of the exciting source fails to reduce the rate of oxidation to that of the unexposed fat. In Fig. 21 is given a set of curves for beef-fat, oxidising at 25° C. in the light

* Probably by destroying inhibitors (page 136).

from a 100 watt tungsten lamp, samples being transferred at intervals to darkness at the same temperature. Oxidation can be seen to proceed in the absence of light at a rate which depends on the amount of peroxide that has already been formed, and which, for

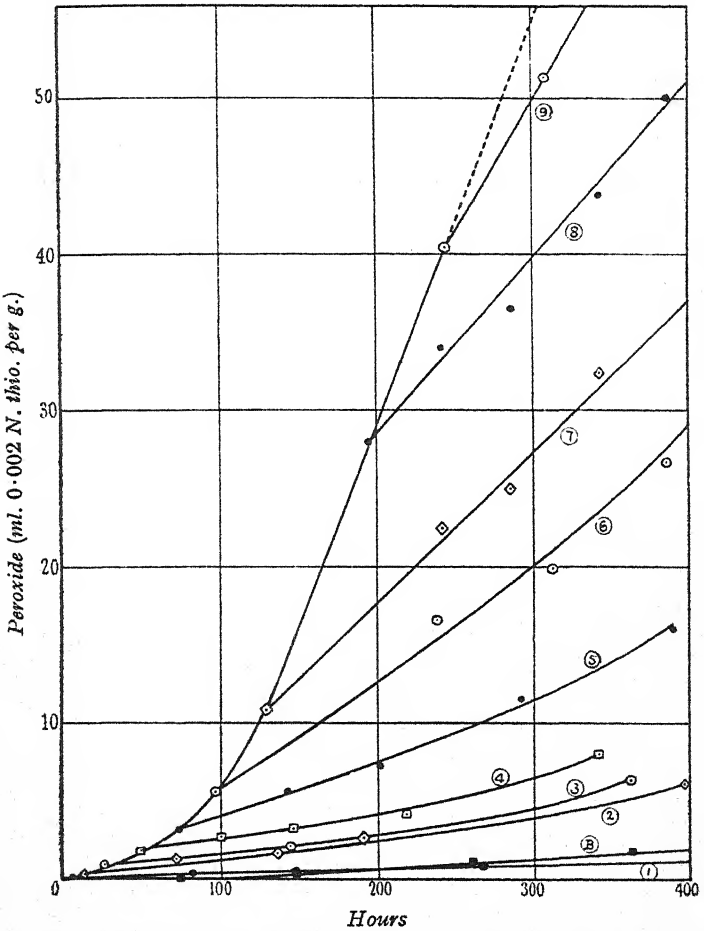


FIG. 21.—*The effect of light in promoting subsequent oxidation of beef-fat. (B) Control in darkness. Samples* 1–9 *transferred to darkness after irradiation.* (Lea[191,192])

very low contents of peroxide, is approximately proportional to the amount present.

It follows from these observations that exposure to light, even of relatively low intensity or for limited periods can have a very pronounced effect in accelerating the development of oxidative rancidity.

Active Wavelengths

The absorption-spectra of fats and oils and of unsaturated fatty acids and esters yield but little information concerning the relative efficiencies of light of different wavelengths in promoting oxidation.

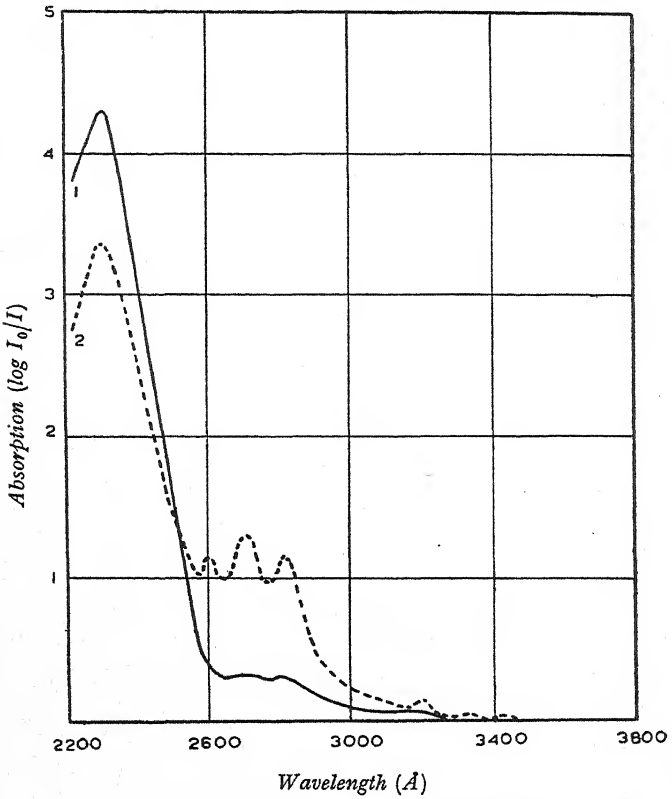


FIG. 22.—*Ultra-violet absorption spectra of* (1) *cottonseed-oil* 1·0% (2 *mm.*) *and* (2) *cottonseed-oil fatty acids* 0·2% (3 *mm.*) (Gillam, Heilbron, Hilditch and Morton[100])

Pure fatty acids, esters and glycerides (with the exception of certain of the highly unsaturated compounds from fish-oils) are colourless, and do not absorb in the visible region. The specific absorption in the visible region shown by the majority of natural fats and oils is due to the presence of pigments, and most of the bands in the near ultra-violet can be traced to sterols, vitamins and other non-fatty substances[39,201]. Extensive general absorption of the very short waves below 2,550 Å occurs (Fig. 22), but is of little practical importance, since the spectrum of sunlight at the earth's surface ends at approximately 2,960–3,120 Å, according to the season. Passage through common green window-glass removes rays shorter

than 3,200 or 3,400 Å*. Free fatty acids show bands at about 2,800 Å, but the glycerides apparently have no selective absorption in this region.[39, 129, 199] Autoxidation markedly increases the opacity of fats and oils to ultra-violet light, the upper limit of general absorption moving towards the visible[201].

Ultra-violet light is extremely effective in accelerating deterioration. Direct sunlight, or light from a clear or lightly clouded sky, is rich in ultra-violet rays. The rate of oxidation of a fat exposed to air and daylight will thus depend largely on the amount of light which can reach it directly from the sky[180]. Most of the light in a ground-floor room at some distance from the window has already undergone reflexion one or more times, and thus lost much of its ultra-violet component. Simple instruments for the measurement of the ultra-violet content of daylight are available[232], or useful results can be obtained by exposure of a piece of photographic printing paper under a suitable filter[213].

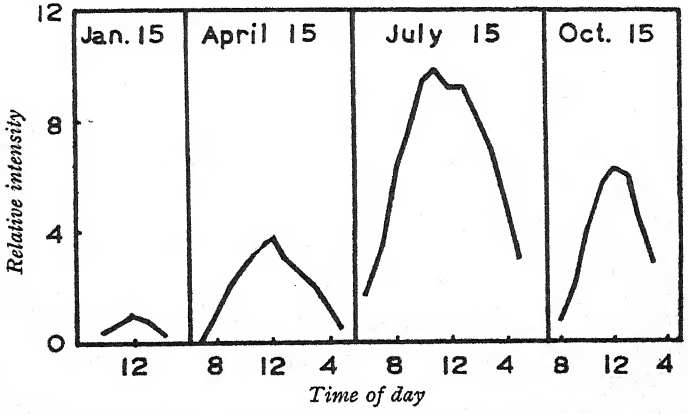


FIG. 23.—*Seasonal variation in the ultra-violet component of sunlight.* (Price[239])

Since the short-wave end of the spectrum is so effective in promoting rancidity, it is obvious that the power of sunlight in this respect must vary considerably with the season. During the winter months in these latitudes the sun never reaches high altitudes and its light, penetrating through a great mass of air carrying dust and smoke, loses a large portion of its intensity at the shortest, least penetrating wavelengths. The result is that the seasonal variation in intensity is much more marked for the invisible ultra-violet light than it is for the visible part of the spectrum (Fig. 23).

But visible as well as ultra-violet light can accelerate the oxidation of fats. Figs. 20 and 21 and other results[191] indicate that marked effects can be obtained by the action of the light from an ordinary tungsten lamp, which radiates very little energy below

* The visible spectrum extends approximately from 4,000–7,500 Å.

4,000 Å[184]. Obviously storage in darkness or the use of opaque wrappers for fat-containing foods will, by the exclusion of all light, completely eliminate spoilage from this cause. In many cases, however, the attractive appearance of goods packed in transparent or partly translucent wrappers, is a decided asset from the salesman's point of view. The question of the relative activities of light of the various colours of the visible spectrum has therefore attracted considerable attention, owing to the possibility of using wrappers tinted to exclude chemically active wavelengths while still retaining a high degree of transparency.

For many years it had been assumed, by analogy with other light-sensitive chemical reactions, that the short-wave, high-energy, blue end of the spectrum would be more effective in accelerating oxidation of fats than the longer yellow and red rays. Thus arose the practice of packing cod-liver oil and many light-sensitive non-fatty substances in amber-tinted bottles, which were usually so dark as to ensure a considerable measure of protection, even in the absence of selective absorption of the chemically active rays. It has been shown, for example, that the tallowy flavours produced in milk or cream on exposure to sunlight, can be prevented by the use of brown in place of clear glass bottles[125].

Coe and Le Clerc[45, 48, 51, 49] exposed numerous fat-containing foods to light transmitted by coloured cellophanes and papers, and by Corning colour-filters. Metallic foils and black papers, which excluded all light, were also used. The progress of oxidation was followed by odour and taste, supplemented in earlier experiments by the Kreis and Schiff tests, and in later work by estimations of peroxide. Wrappers excluding all light gave the greatest protection. Of the others, " sextant " green, transmitting between 4,900 and 5,600 or 5,800 Å, and very dark red, transmitting only slightly in the visible spectrum but freely in the infra-red, were almost as good. These delayed rancidity for very much longer periods than did filters passing red, orange, yellow, blue, violet or ultra-violet rays. Blue-green and yellow-green were much less effective than " sextant " green. No attempt was made to control the intensity of the light reaching the fat*. From these results it was concluded that only green and infra-red rays are inert as far as the production of rancidity is concerned. Patents have been taken out to cover the commercial packing of food-products in wrappers of the special green[47]

In one experiment[48] butter was exposed for 17 hours to monochromatic light (intensity apparently not controlled) of wavelengths 3,020, 3,130, 3,650, 4,060, 4,358 and 5,461 Å. Rancidity developed in all cases except the last, which is within the green. No tests were made with light of longer wavelength. These results

* Carpenter [38], who found a green Corning filter effective in protecting fruit-juices against the deleterious effects of sunlight, pointed out that its maximum transparency was only about 20 per cent., as compared with 70–80 per cent. for all the other filters examined.

can therefore hardly be taken as evidence of any particular virtue in the green, since they would agree equally well with a chemical activity decreasing with increasing wavelength to a very low value somewhere above 4,358 Å.

Tables of peroxide-values quoted in later papers[49, 46] also do not seem entirely to support the conclusions drawn, in that the effects produced by filters opaque to rays below 4,700 Å appear to be relatively small and of the same order of magnitude whatever the colour. Conversely, filters which transmit appreciable quantities of rays shorter than 4,500 Å produce a larger effect. The peroxide-values afford evidence of oxidation but do not, of course, bear the same relationship to flavour in the case of oils exposed to strong daylight (south window) and to darkness. In this respect Coe and Le Clerc's observation that rancidity appears at lower peroxide-values in the light than in darkness, confirms earlier results (page 115). The case presented by these authors for the special use of green, rather than of any colour which does not transmit below about 4,700 Å, does not appear to have been proved.

Holm and Greenbank, in a paper read before a section of the American Chemical Society in 1929, but only published[141] after the appearance of Coe and Le Clerc's results, described experiments in which cottonseed-oil was irradiated through colour-filters, each transmitting only one band of wavelengths, approximately 500 Å wide. The volume of oxygen absorbed per 100 ml. of oil during an exposure of eight hours was measured. In a second experiment the relative rates of reduction of methylene blue mixed with the oil were used as a measure of the oxidation produced by light transmitted by glasses of different colours. In all cases the results were corrected to refer to light of equal intensities. Both sets of data agreed in placing the region of maximal effectiveness in the yellow-orange. A high value found for green glass in the second experiment was attributed to the transmission of active yellow and orange rays. The light least effective in accelerating oxidation was stated to be in the blue. These results, though obtained by an apparently satisfactory technique, are in complete opposition to the more recent work in this field.

Davies[61, 67] has examined a number of coloured cellophanes and other wrapping materials for (*a*) transmission of light, (*b*) chemical activity of the transmitted light, as measured by ability to bleach methylene blue dissolved in acetone, in sodium oleate solution or in milk, and (*c*) the degree of protection afforded to fats and fat-containing foods exposed to sunlight. His conclusions are summarised below.

Sunlight transmitted by thin white glass was as effective as direct light. The wrappers used could be divided into three classes, according to the chemical activity of the light transmitted by them. Class I, which absorbed very little of the active light, consisted of colourless, pale blue, pink, orange, and lemon cellophanes,

and transparent paper. All of these materials transmitted a considerable amount of blue light between 4,000 and 5,000 Å, and permitted nearly as rapid autoxidation as direct exposure. Class II, which showed appreciable absorption of the active light and gave some protection, contained heliotrope and light green cellophanes and greaseproof paper. Class III possessed high protective properties and showed powerful absorption of the chemically active ultra-violet and blue rays. Included in this class were all the deeply coloured cellophanes—blue, green, brown and red—irrespective of colour. Sunlight passing through these for 40 hours produced no appreciable increase in the peroxide-oxygen of the fat of biscuit-meal.

Davies therefore concluded that, for preservation of the fat of foods in coloured cellophane, it is on the whole the depth of colour and not the actual tint which is of importance.

The recent work of Morgan[213] is in general agreement with that of Davies as regards the active wavelengths of light. In Morgan's experiments potato-chips in small bags made of coloured transparent cellulose were exposed either to direct sunlight, or on a rotating table beneath a General Electric sun-lamp. The energies radiated by this lamp in the ultra-violet (2,800–4,000 Å) and in the visible (4,000–7,600 Å) were in the ratio of 14·5 to 85·5 per cent., as compared with the corresponding figures of 10·3 and 89·7 per cent. for high-altitude sunlight. Exposure for 2·5 hours under the lamp was roughly equivalent to one day in direct sunlight.

Several hundred cellulose films, covering a wide range of shades and depths of colour, were compared for "rancidity-retarding value," defined as the ratio of the lengths of time necessary for the development of rancidity under coloured and colourless films illuminated simultaneously. Uncoloured, transparent films had no appreciable effect in retarding rancidity. The efficiencies of the

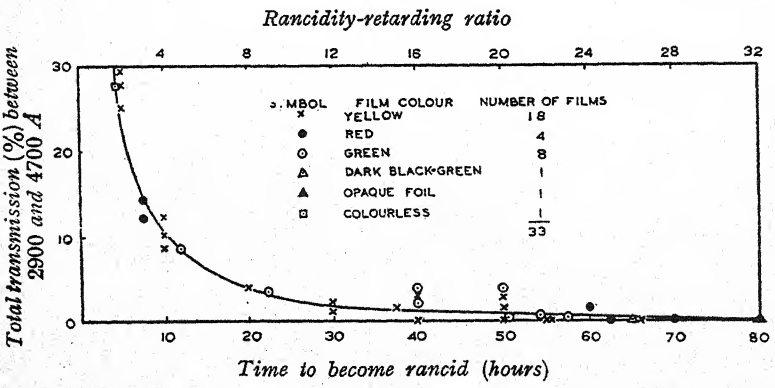


FIG. 24.—*Dependence of production of rancidity on transparency in the ultra-violet and blue regions of the spectrum.* (Morgan[213])

others seemed to be correlated, not with the colour of the film, but with its absorption of the ultra-violet and blue rays. From the data obtained it was concluded that the effect of blue light increased rapidly with decreasing wavelength, ultra-violet rays between 4,000 and 2,900 Å being still more effective. In opposition to the results of Coe and Le Clerc and of Holm and Greenbank, the quantity of light other than ultra-violet or blue (i.e., of wavelength longer

TABLE 37.—*Relative efficiencies of coloured films in retarding rancidity.* (Morgan[213])

Colour of film	Transmission (%)					
	Ultra-violet and blue (2900–4900Å)	Green (4900–5300 Å)	Yellow and orange (5300–6500 Å)	Red (6500–6900 Å)	All rays (4900–6900 Å)	Rancidity-retarding ratio
Yellow D	27·6	48	75·9	87·3	74·6	2
Colourless R ..	27·4	90	90	90	90	2
Green I	15·8	48	24·3	26·5	29·5	3
Red A	10·6	0·7	61·1	90	57·9	4
Yellow E	9·4	60	90	90	84	4
Yellow F	4·1	42·5	88·2	90	80·4	8
Opaque green J ..	3·3	15	9·7	4·8	9·8	10
Green N	3·6	85·7	12·9	0	11·5	12
Yellow O	0·8	39·1	85·7	90	77·2	15
Yellow O.G. ..	0·2	33	74·5	81·6	68·3	20
Green K	1·2	31·6	15·6	40	23·7	22
Yellow H ..	0·8	28	80·7	90	72	22
Orange-yellow ..	0	1·3	72·2	90	61·6	22
Red B	1·5	0	50·1	90	48·1	24
Green L	0·5	9	11	0	8·4	25
Yellow P	0·2	19	75	90	66·8	26
Opaque-black-green M ..	0·1	7·4	7·5	4·5	6·9	26
Red C	0	0	32·4	89	37·2	28
Opaque foil ..	0	0	0	0	0	32

than 4,900 Å) reaching the fat appeared to have very little effect on the development of rancidity (Table 37). Fig. 24 shows the percentage of ultra-violet *plus* blue light (2,900–4,700 Å) transmitted by films of several colours, plotted against the rancidity-retarding value found experimentally. The transmission of small amounts of active light greatly reduced the time necessary for the development of rancidity. Calculation from these data shows that the rates of deterioration were roughly proportional to the intensities of the active light reaching the fat.

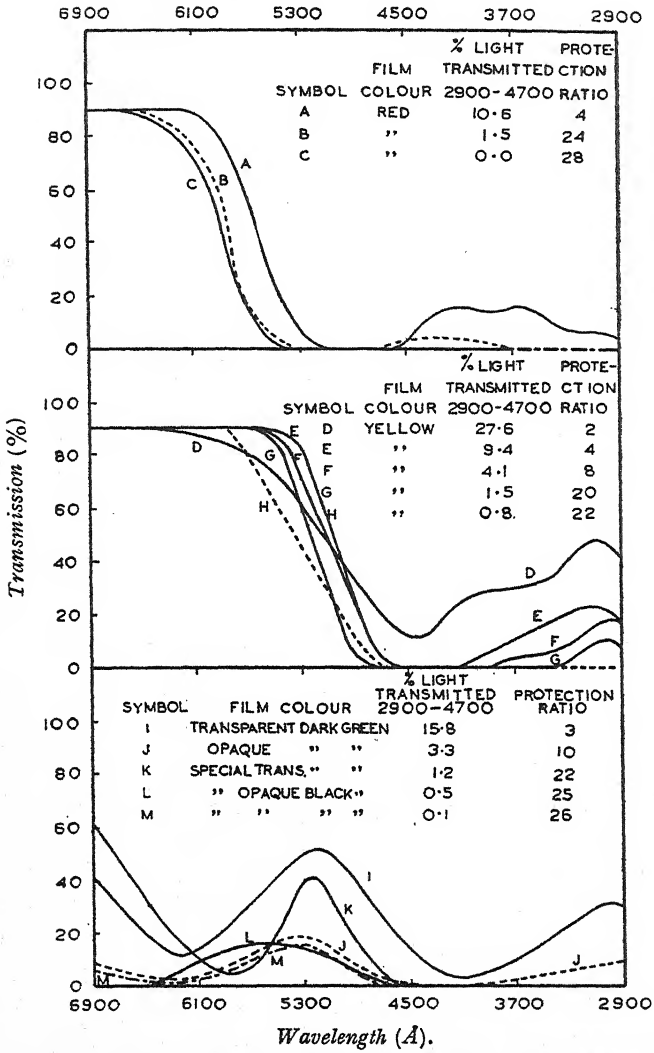


FIG. 25. *Transmission-curves and protection-ratios for red, yellow and green films.* (Morgan[213])

Morgan points out that, since ultra-violet light is invisible and the eye relatively insensitive to blue light,* the utility of a coloured wrapper for the purpose of retarding rancidity cannot be gauged by visual inspection. Films of red, orange, yellow or green colour can be obtained which vary in rancidity-retarding value over almost the whole range from the colourless film to the opaque foil (Fig. 25).

* The eye is most sensitive to yellow light.

Summary.—The publicity which has attended some of the recent work on the effect of light on fats has probably caused some misapprehension regarding the scope and importance of this factor in the production of rancidity. Exposure to light, though of undoubted importance, is only one of the factors which influence the development of oxidative rancidity. Statements which infer that spoilage does not occur in the absence of light, and that copper or iron without light will not produce rancidity[46], are incorrect. Obviously, too, spoilage by light is limited to those cases in which products are exposed to its influence during preparation or marketing. No possible advantage is to be gained, for example, by using green wrappers for containers which are already perfectly opaque, as has sometimes been suggested! Nevertheless, many cases do exist in which foods are exposed for considerable periods to air and light, either directly or within packages which are not impermeable to light, and in these, access of the chemically active rays will produce abnormally rapid spoilage. As examples of such materials may be quoted butter, margarine, lard and shortening fats, salad-oils and dressings, biscuits, cakes and pastries, sweets and chocolate, nuts, peanut-butter, potato-chips, cereals and flours, milk, sliced bacon and coffee.

The effect of light in such cases can be eliminated by use of outer wrappers of metallic foil or of other opaque material. Alternatively, a dark chocolate-brown wrapper which passes very little light may sometimes be more suitable. If it is desired to retain an appreciable measure of transparency, a coloured cellophane or paper which absorbs most of the chemically active wavelengths may be used. All authorities agree in regarding the ultra-violet (2,900–4,000 Å) as the most harmful part of the spectrum,* and the experimental evidence strongly supports the view that the blue rays of wavelengths shorter than about 4,700 or 5,000 Å constitute the most effective portion of visible light. A wrapper which excludes all light below 4,700–5,000 Å will therefore afford a very large measure of protection.

Such a wrapper may be yellow, brown or red in colour, and can transmit so freely at wavelengths above 5,000 Å as to pass over 60 per cent. of the total visible rays. Yellow films have the advantage of transmitting the highest possible proportion of non-active light, and arrest only the blue and part of the green, so that colours other than these will appear very little altered by inspection through the film. Orange and red films are, of course, equally effective, but transmit less total light, causing yellow, as well as green and blue, to appear dark by inspection through them. According to Davies, green or even blue wrappers can be used, but must be dark

* Patents have been taken out in Germany and France covering the use of cellulose wrappers rendered opaque to ultra-violet light by impregnation with colourless organic compounds. Vegetable parchment and grease-proof paper, even uncoloured, absorb a considerable proportion of the actinic light[67].

and very dark respectively in order to exclude the active light. Green has the advantage of absorbing the yellow and red rays which have been stated to produce rancidity, though the balance of the evidence at present available is against this conclusion. Dark green, through which any colour other than green appears almost black, does not seem an attractive shade for transparent or translucent wrappers for foodstuffs.

There is every probability that protective coloured wrappers and glass containers will come into increasing use in the packing of certain classes of food products.

Other Effects of Light

Oils and fats which have been exposed to light in the presence of air are found to have acquired the property of fogging a photographic place held at some little distance from the surface of the sample. This phenomenon, usually referred to as the "Russel effect," is not peculiar to fats, but has also been observed in many other autoxidisable substances. The wavelengths of light concerned appear to be those of the ultra-violet and blue regions of the spectrum, which have already been shown to be responsible for the oxidation of fat[281]. The effect on the plate is usually considered to be due to hydrogen peroxide evolved during oxidation of the fat. Both this substance and ozone act on the silver salts of the photographic emulsion in a manner similar to that of light. Other theories which have been advanced suggest that the Russel effect is due to butyric acid[306], or to ultra-violet re-radiation from the fat[268]. The theory of secondary ultra-violet radiation has been used to explain the germicidal action observed in the vicinity of oils which have recently been subjected to irradiation, and which subsequently persists in many cases for long periods. It is more probable, however, that this action is also due to volatile peroxides[128].

Chemical Accelerators of Oxidation

Peroxides

It has already been pointed out that the products of oxidation have a marked effect in reducing the induction-period of fresh fats, and that this action, in major part at least, is due to a destructive effect on the inhibitors present (page 136). Perbenzoic acid, which is a powerful pro-oxidant for lard[227], has been stated to have only a very slight effect on purified oleic acid, presumably almost free from inhibitors[123]. It is uncertain whether any additional direct acceleration of oxidation occurs.

The active constituent of oxidised fats is usually assumed to be the fat peroxide. Certainly the addition of peroxides other than those of fats, *e.g.*, oxidised turpentine or hydrogen peroxide, can be equally effective, and organic peracids are well known as accelerators of autoxidation. An actively oxidising fat must contain, in addition to the ordinary fat peroxide, relatively small quantities of substances

much more effective in destroying natural inhibitors. These are the recently formed molecules of peroxide which have not yet given up their energy of formation, traces of peracids derived from autoxidation of the gradually accumulating aldehydes, and the labile peroxides to which reference has already been made (page 91). It is these reactive peroxides which are probably responsible for a large proportion of the pro-oxidant effect of oxidised fats, and for the apparently autocatalytic nature of the oxidation of fat. There is some evidence, however, that non-volatile, non-peroxidic products of oxidation of high molecular weight may also function as accelerators[253].

As oxidation proceeds, progressively larger quantities of a particular antioxidant are found to be required to arrest the reaction for a standard length of time[307, 92]. Since the total peroxide-content is only slightly depressed by the addition of the inhibitor, it must be the small but increasing amounts of highly reactive peroxides that are mainly concerned in destruction of the inhibitor.

It is of interest to note that, in the case of the autoxidation of benzaldehyde in the presence of anthracene, a somewhat similar reasoning has led to the conclusion that here also the peroxide formed as the first product of the reaction is not a single substance, but a mixture of small quantities of more reactive with a larger quantity of a less reactive peroxide, the *latter* being probably perbenzoic acid[2, 30].

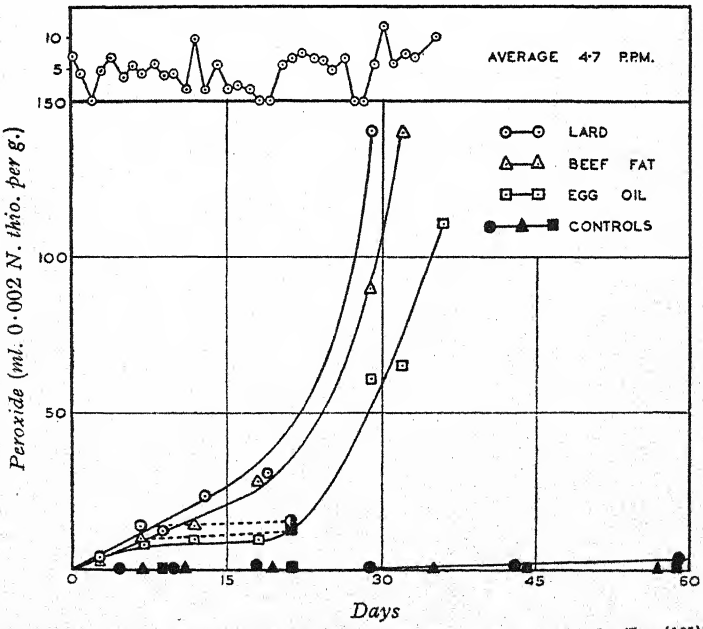


FIG. 26.—*The effect of ozone on the oxidation of fats at* 0° *C.* (Lea[188])

Ozone

The action of ozone on fats is of some importance, since this gas in low concentration is frequently used for removing odours from stores and ships' holds (page 41), and even to some slight extent as an inhibitor of the growth of micro-organisms (page 71).

Ozone, of course, attacks unsaturated fats with the formation of ozonides, which subsequently decompose in a manner very similar to that of the peroxides produced in oxidative rancidity. Moreover, ozone is a powerful oxidising agent and a catalyst for autoxidative reactions (*e.g.*, that of ethylene, etc.), properties on which its deodorising activity largely depends. It is therefore not surprising to find that the susceptibility of pure fats to oxidative rancidity is greatly increased by exposure to ozone.[200, 195] Fig. 26 shows the effect of ozone at approximately 4·7 parts per million for five hours per day on the induction-periods of lard, beef-fat and egg-oil, dispersed in thin films and stored at 0°C. In these cases the characteristic " cucumber " as well as a tallowy odour was clearly perceptible after 12 days. The controls, on the other hand, were still sweet after storage for three months.

Various other inorganic oxidising agents, *e.g.*, hydrogen peroxide and nitrous acid[189], accelerate the oxidation of fats, the latter being a particularly powerful pro-oxidant. A number of organic substances, *e.g.*, nitro-compounds and aromatic aldehydes are also pro-oxidants[222].

The Action of Metals

The pronounced effect of certain metals, particularly copper and to a smaller extent iron, in producing " off " flavours in dairy-products has been known for over 30 years[101], but only comparatively recently has attention been directed to this subject in other branches of the food-industry. The relative activities of the various common metals and alloys in accelerating the development of rancidity is obviously a matter of considerable practical importance, since it affects the choice of material for the construction of food-processing plant, storage-vessels and containers. The present state of knowledge concerning the behaviour of metals is far from satisfactory, and only a brief survey of the more outstanding points will be given here.

The effectiveness of a particular metal or alloy in accelerating the production of rancidity (" tallowiness ") in the fat of food-materials brought into contact with it, will depend upon several factors (a) the possibility of a catalysed oxidation of the fat at the surface of the metal, without appreciable solution of the latter, (b) the ease with which the metal is attacked and dissolved under the conditions of use, and (c) the magnitude of the effect of the metal, once it has been brought by solution into intimate contact with the fat.

Investigations on the effect of metals fall into three classes. In most of the earlier and some of the more recent work, strips of metal were immersed in the fat exposed to air, and the rate of oxidation compared with that of control samples in the absence of the metal. Empirical experiments of this type approximate fairly closely to practical conditions, and assess the combined effect of surface activity, solubility, and accelerating effect of the dissolved metal. In a few cases the degree in which metals and alloys are attacked or corroded by foodstuffs under various conditions has been investigated separately. In others data have been collected concerning the influence of small quantities of metals, added in soluble form to fats, or as soluble salts to an aqueous phase in contact with fat.

Relative Activities of Metals

Cases of oxidative deterioration in fat-containing materials of all kinds are frequently traced to contamination with copper and iron. Rancidity and discoloration in soaps, for example, may be due to traces of these metals, derived from the steel or brass dies of stamping machines[318, 261]. Powdered soaps[269, 7] and textile oleins dispersed on fibres[282, 84] have been known, when contaminated with these metals, to oxidise so rapidly as to char or ignite spontaneously.

In Metallic Form.—As early as 1905, Golding and Feilman[101] observed that milk passed over a tinned copper cooler from which part of the plating had been worn away in use, acquired a tallowy odour on keeping. Contact with copper or brass or, in a less degree, with iron, was also found to be effective in producing tallowy odours and flavours in butter[152]. Subsequently an attempt was made to place a number of the metals employed in the equipment of creameries in order of desirability, using as criteria the solubility of the metals in weak organic acids and their effect in producing "off" flavours in milk-products[151]. The metals examined were thus divided into four groups: (*a*) allegheny metal, tin and heavily tinned copper; (*b*) nickel, aluminium and manganese-aluminium alloy; (*c*) monel metal, enduro, ascaloy and nickel silver; (*d*) tinned iron, copper, galvanized iron, iron and zinc. Measurement of the length of time necessary for fresh corn- and cottonseed-oils and lard to become rancid (odour and Kreis test) when strips of various metals were immersed in them, indicated that copper was the most, and tin and aluminium the least, active of the metals studied. Lead, iron and zinc were intermediate in activity[82].

In Solution.—Salts of copper, iron, nickel, cobalt, chromium and manganese have been found to produce oxidation ("tallowiness") when incorporated into butter at concentrations of from two to 50 parts per million. Copper was much more effective (of the order of 10–12 times) than any of the others, two parts per million being sufficient to produce incipient tallowiness after storage for six days at

room temperature. Tin and aluminium were inactive, even at a concentration of 100 parts per million[60, 62].

Metallic oleates (0·01 per cent.) accelerated the bleaching (oxidation) of vegetable oils at 80° C. in descending order of efficiency as follows:—cobalt (4), manganese (5), copper (9), nickel (15), lead (16), iron (17).* Acceleration of the oxidation of linseed: and of olive-oils, as measured by the length of time required for the oil containing 2 per cent. of metallic soap to attain a temperature of 200° C. in the Mackey Cloth Oil Tester, placed the metals in a very similar order:—cobalt, manganese, nickel, cerium, lead, chromium, iron, uranium, bismuth, silver, zinc, thorium, mercury, aluminium and tin, the last seven being much less active than the others[204]. Metallic linoleates have been found to accelerate the oxidation of herring-oil in glacial acetic acid, in the following order:—vanadium, cobalt, copper, manganese, iron and lead, vanadium and cobalt being much more effective than any of the others[302]. Several of these metals, e.g., cobalt, manganese and lead, usually in the form of linoleate, resinate, or naphthenate, are used extensively as driers for reducing the drying time of oil-paints, cobalt being the most active[90, 74]. Cobalt is also stated to be much more effective than copper or iron in catalysing the oxidation of linoleic acid[91].

The addition of a soluble copper salt (stearate) at a concentration of 1-2 parts per million greatly increased the rate of formation of

TABLE 38.—*The effect of metals on the induction-period of lard at 98° C.*†

Metal sheet		Metal in solution				
Order of activity	Induction-period of lard (hours)	Order of activity	Concentration‡ to reduce induction-period from 16 hours to			
			12	8	4	2
Copper ..	1·0	Copper	—	—	—	0·15
Lead ..	1·2	Manganese	—	0·6	1	1·8
Zinc ..	1·7	Iron+++	—	0·6	2·5	10
Tin ..	2·5	Vanadium	—	2·4	4	10
Iron ..	5·0	Chromium	—	1·2	5	—
Aluminium	7·5	Nickel	1	2	7	—
—	16·0	Zinc	7	20	—	—
		Aluminium	20	>40	—	—
		Lead	20	—	—	—
		Tin++++	>40	—	—	—

† From data given by King, Roschen and Irwin[169].
‡ Parts per million.

* The figures in parentheses indicate the numbers of hours necessary for bleaching an oil which required 20 hours in the absence of the metal[242].

peroxide in lard at 100° C.[168, 256] Copper and manganese were effective in reducing the fading time of methylene blue when fat containing the dye was exposed to light (page 129), five parts per million of copper producing approximately the same effect as 50 parts per million of manganese. Under these conditions ferrous iron and tin were inactive, and zinc slightly protective[255]. Measurement of the effect of metals (as lactates) on the induction-period of butter-fat at 100° C. by the oxygen-absorption method, showed that vanadium and copper, at a concentration of one part per million, were very powerful, and approximately equally active. Iron was much less effective and nickel only slightly so, while zinc again appeared slightly to retard oxidation[31].

King, Roschen and Irwin[169] conducted a series of experiments in which they determined the effect of a number of metals, both in sheet and in solution (as stearate), on the induction-period of lard at 98° C., as measured by the peroxide method. The results (Table 38) classify the metals in different orders according to whether in the solid state or in solution in the fat. Tin, for example, invariably inert in solution, appears from these data to be an effective surface catalyst, while iron, which is active in solution, is apparently relatively inert in the solid form. Of the six metals used in the sheet, only one (lead) suffered detectable loss in weight. The conclusion was therefore reached, in confirmation of the earlier findings of Emery and Henley[82], that some metals are capable of accelerating oxidation of fats without being attacked or oxidised themselves.*

Action of Copper and Iron in Aqueous Solution

With the exception of a few experiments on milk and butter, practically the whole of the published work has been on the direct addition of metals or metallic salts to dry fats, whereas most foodstuffs contain, in addition to the fat, an aqueous phase containing non-fatty material.

In some recent work Lea[189] has studied the effect of metallic salts dissolved in an aqueous phase in contact with a fat. Extremely minute amounts of copper (one in 100 million) had a detectable influence on the rate of oxidation, which increased rapidly at first with increasing concentration of the metal (Fig. 27), but later appeared to approach a limiting value (Fig. 30). Protein, cyanide and other water-soluble antioxidants completely inhibited the pro-oxidant effect of small amounts of copper (<1 p.p.m.), but even 4 per cent. of protein failed to prevent a powerful acceleration of oxidation by larger quantities of the metal. Iron in organic combination (hæmoglobin, met-hæmoglobin and hæmin) has been shown to be effective in accelerating the oxidation of linseed-oil at 37° C. and

* But it should be noted that the loss of only 0·1 mg. of metal would correspond to a concentration of five parts per million on the 20 ml. of fat used. Lead dissolved to a concentration of 500–1000 parts per million.

in this state is not inhibited by cyanide[249] In acid solution iron is approximately one-twentieth as active as copper. In neutral or alkaline solution, i.e., colloidal ferric hydroxide, the metal is inactive at a concentration of five parts per million[189].

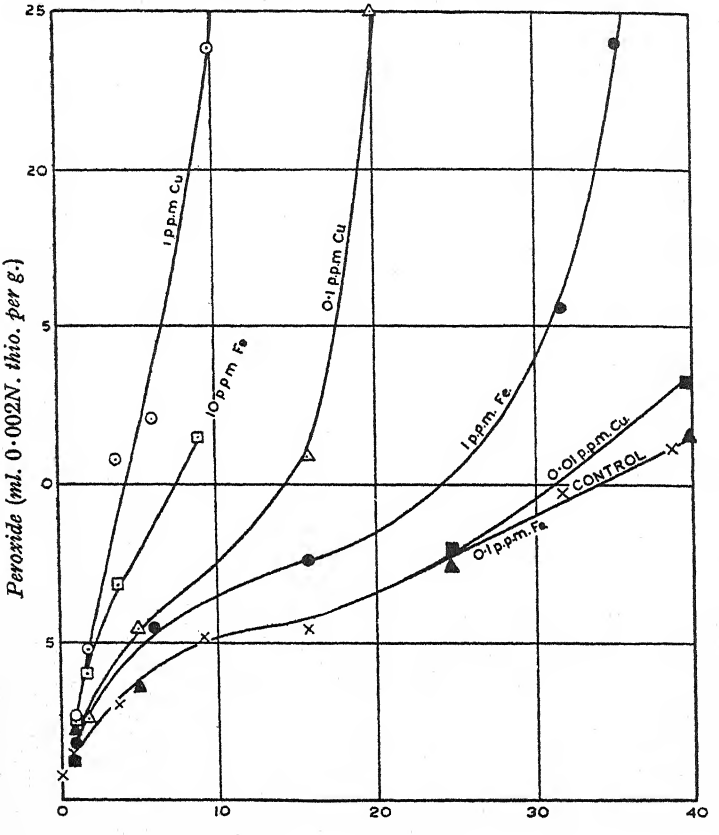


FIG. 27.—*The autoxidation of herring-oil in contact with an aqueous phase containing copper or iron in solution.* (Lea[189])

Suitability of Metals for use with Fats

The general conclusion suggested by the experimental data summarised above is that most of the common metals, when present in solution in a fat, accelerate the appearance of oxidative rancidity. Cobalt, copper and, in some cases, vanadium are the most active, followed by iron and manganese, and nickel and chromium. The least effective are tin and aluminium. Zinc in solution appears to be relatively inert, but its ready solubility in dilute acids and the rather toxic nature of its salts renders the use of zinc or of galvanized iron

undesirable. Lead, for similar reasons, is still less suitable for contact with the fats of food.

Metals and alloys for use with fatty materials should be (*a*) highly resistant to corrosion under the particular conditions for which they are required, e.g., the nickel-chromium steels of the " staybrite " type[283, 212], (*b*) if not completely resistant to corrosion, the traces of metal dissolved should be relatively inactive, e.g., tin or aluminium. The selection of metals for the construction of plant for processing food is further complicated by the fact that where, as is often the case, more than one metal is employed, contact-corrosion may lead to rapid solution of a metal which alone would remain practically unattacked.

Very little is yet known regarding surface activity. The stainless steels mentioned above have proved very satisfactory in the dairy industry, and yet King, Roschen and Irwin[169] found that a steel of this type accelerated the oxidation of dry fat at 98° C. almost as much as sheet iron. Similarly, tin, which has been used successfully in contact with milk,* is apparently a fairly active surface catalyst for the dry fat (Table 38). Copper and its alloys should, of course, be avoided in all cases unless *completely* protected by a heavy coating of tin. The question of metallic corrosion and tainting by metals is further mentioned in connection with dairy products (page 203).

Mechanism of the Action of Metals

The harmful effect of metals on fats is due to an acceleration of the normal process of oxidation; in the absence of oxygen they are unable to produce rancidity. A trace of an active metal added to a fat will reduce, and may almost destroy, the induction-period, but some doubt exists concerning the effect on subsequent oxidation.

The maximal rate of absorption of oxygen by linseed-oil at a given temperature has been said to be almost, if not completely, unaffected by the presence of catalysts or inhibitors, metals simply displacing the region of maximal slope along the time-axis towards the origin, and inhibitors causing a displacement in the opposite direction[279]. Rates of absorption have also been observed for particular temperatures beyond which addition of further amounts of metallic catalyst produces no effect (Table 35). In such cases, however, it is possible that the rate of oxidation has been limited by some external factor, such as the rate of diffusion of oxygen into the oil

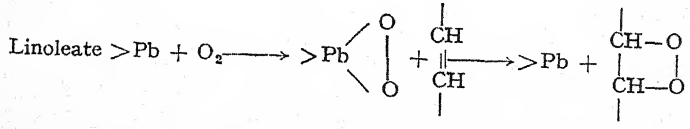
Yamaguchi[320] has recently found that copper oleate and manganese linoleate slightly *reduce* the rate of disappearance of the double bonds in oils after the end of the induction-period, and therefore concludes that these metals slightly *retard* the initial combination of the fat with oxygen. The copper salt, however, powerfully

* In milk and other products containing water, protein, carbohydrate, salts, etc., in addition to fat, the surface activity of metals may be much reduced by the formation of protective coatings, which also reduce loss by solution.

catalysed decomposition and further oxidation of the fat peroxides, resulting in an *increased* rate of absorption of oxygen and the evolution of carbon dioxide. This latter observation is in agreement with others to the effect that active metals reduce the stability of titratable peroxides[256], and prevent the evolution of hydrogen peroxide from oils after exposure to light[281].

The evidence at present available therefore indicates that metallic pro-oxidants (1) powerfully catalyse oxidative decomposition of the natural inhibitors present, resulting in the reduction or elimination of the induction-period, (2)* have little effect on the rate of the subsequent more rapid oxidation, and (3) promote the decomposition and further oxidation of the fat peroxide. The use of cobalt and similar metallic driers in paints has thus the disadvantage that, in addition to reducing the drying time, they also tend to accelerate subsequent oxidative disintegration of the dried film. At temperatures above 75°C. even pure saturated fatty acids react with oxygen in the presence of a metallic catalyst[77], and at lower temperatures the presence of a metal will probably assist the reactive peroxides to attack the hydrocarbon chain of both saturated and unsaturated acids.

There seems to be no simple relation between the chemical properties of metals and their activities as accelerators of oxidation, but the generalisation of Mackey and Ingle [204] is useful as a rough guide. According to these authors, " In its oil-soluble form a metal which exists in more than one state of oxidation acts as a drier or oxygen carrier, provided that the salts of the lower oxides are more stable than the higher. The more oxides a metal can form the greater its catalytic power." Ingle[155] suggests in the case, for example, of lead, that the metal is alternately peroxidised and reduced, the peroxidised lead compound actually oxidising the fat (or anti-oxidant).

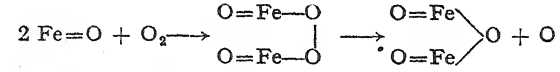


Similarly, Hyman and Wagner[153] have pointed out that cobalt(ous) oleate itself oxidises with a pronounced induction-period, and suggest that the active substance involved is actually a complex oxidised cobalt(ic) salt. In oxidations induced by cerous salts, an unstable intermediate peroxide (Ce_2O_5) is known to be formed.

The effect of iron compounds in catalysing autoxidative reactions has been extensively studied, usually from the view-point of biological systems. In reactions of this-type it is assumed that oxygen

* The evidence on which (2) and (3) are based has been obtained mainly at high temperatures. It is possible that these statements may need modification for lower temperatures, when the thermal reaction is slow.

is activated by the formation of an unstable peroxide of iron, e.g. Fe_2O_4[122]



This may then react as shown above for lead or, according to Wieland[317], Fe_2O_4 in oxidising the reacting substance may be reduced to Fe_2O_3. In this case some mechanism for reducing the Fe_2O_3 back to FeO must be present, or the reaction will stop. Substances oxidisable by Fe^{+++} but not by molecular oxygen, will thus be oxidised by oxygen in the presence of an iron salt. When peroxides are present the intermediate iron peroxide may be FeO_3[122]. The oxidation of ferrous to ferric salts is itself an autoxidative reaction catalysed by copper.

Oxidase Systems

Mention has already been made of oxidase systems as catalysts of the atmospheric oxidation of fats (page 52). This is a relatively new branch of the subject, and little work has yet been done. The few results available seem to indicate that heat-labile organic oxidative catalysts (lipoxidases) may be fairly widely distributed in both the animal and the vegetable kingdoms. They have definitely been shown to be present in the soya and certain other beans, in wheat-germ and peanuts (probably), in milk, in the adipose tissue and muscle of the pig, and in the muscle of the herring. The influence of these enzymes on the development of rancidity has not been examined in detail, but there can be little doubt that under suitable conditions lipoxidases can function as very powerful accelerators of oxidation. Data recently collected concerning the lipoxidases of milk, meat and fish are given in Part VI.

The possibility thus arises of increasing the stability of the fat in certain food-products by heating for a brief period at a temperature sufficiently high to destroy the enzyme. Frequently, however, such treatment will not be practicable. Preliminary results obtained for the lipoxidase of the muscle of pork (page 216) are of interest, in that they indicate a possible alternative method of control by adjustment of *p*H, which may prove applicable in some cases.

As already stated, certain micro-organisms undoubtedly elaborate enzymic systems which are capable of increasing the rate of development of oxidative rancidity in fat (page 53), and cases of spoilage of food may perhaps sometimes be attributable to this cause.

Much further work, however, is necessary on the lipoxidases of tissues and of micro-organisms in order to ascertain the importance of these factors in the development of rancidity in specific food-products, and to work out methods of control.

Chemical Inhibitors of Oxidation.

Moureau and Dufraisse[217, 216] investigated the effect of traces of foreign substances on the velocity of a great many autoxidative reactions. Their results, together with those of other workers, showed that the reaction between an autoxidisable substance and oxygen can be delayed for long periods by the presence of extremely minute quantities of foreign substances, variously termed inhibitors, antioxidants or antioxygens.

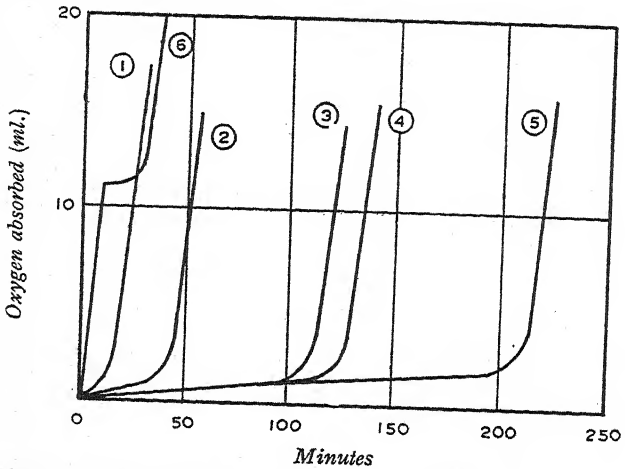


FIG. 28.—*The autoxidation of benzaldehyde at 25°C in the presence of an inhibitor (anthracene) at the following molalities:* (1) 0·000040, (2) 0·000080, (3) 0·000132, (4) 0·000140, (5) 0·000200, (6) 0·000200 *added* 11 *minutes after commencement of the oxidation.* (Branch, Almquist and Goldsworthy[30])

The action of such substances consists essentially in delaying the oxidation by the introduction of an initial lag or induction-period during which oxidation is very (sometimes immeasurably) slow. On the conclusion of the induction-period the rate of oxidation is usually identical with that of the substance in the absence of the inhibitor, as shown in Fig. 28 for the autoxidation of benzaldehyde in the presence of anthracene. Curves of this type show a striking resemblance to those of the oxidation of natural fats, but it is only within the last few years that the induction-periods of the latter have come to be regarded as due to the presence of traces of natural inhibitors.[131, 208, 320]

Natural Antioxidants

The fact that the oils present in ripening seeds and nuts, and in other plant and animal tissues exposed to air and light do not become rancid, points to the existence of some mechanism which protects them from oxidation, either by maintaining the concentration of oxygen in contact with the fat at an extremely low level, or by the formation of substances which inhibit oxidation. The first

experimental indication of the presence of such inhibitors in certain oils appears to have been the observation that oxidative destruction of the fat-soluble vitamins A and (especially) E in experimental diets was greatly retarded when animal fats, such as lard and cod-liver oil, were replaced or supplemented by vegetable oils of equal or greater unsaturation[5, 205] Subsequent work has demonstrated the association of these vitamins in a number of plant tissues with inhibitors which prolong the induction-period of fats, and provides some support for the theory that the vitamins (and carotene) owe their survival in an environment otherwise favourable to oxidation to the protection afforded by antioxidants.[55, 231, 29] Further evidence has been provided by the observation that the glycerides resynthesised from the constituent fatty acids (after distillation) of several natural oils are very much less resistant to oxidation than the oils themselves[135], thereby suggesting that inhibiting substances are present in the original oils.

TABLE 39.—*Effect of refining on the susceptibility to oxidation of corn-oil.*

(Mattill and Crawford[208])

Oil	Induction-period (hours)
(1) Solvent-extracted oil (petroleum ether, below 50° C.)	40·0
(2) Oil expressed at 93° C.	10·5
(3) No. 2, neutralised at 46°–66° C. for 1 hour	8·3
(4) No. 3, treated with fuller's earth at 60° C. and filtered (*in vacuo*)	7·0
(5) No. 4, " winterized " by holding at —1° C. for two days and filtering	7·0
(6) No. 5, deodorized by heating *in vacuo* for 5 hours ..	5·0

Destruction of the natural antioxidants during the ordinary processes of extraction and refining leads to a very pronounced reduction in potential resistance to oxidation (Table 39), alkali, bleaching agents and aeration at high temperatures being particularly destructive of natural inhibitors. Even boiling with " air-free " water effects a marked reduction in the potential keeping properties of some oils, at least 0·03 per cent. of hydroquinone being necessary to restore the induction-period of olive-oil to its original value after this treatment[15].

Mattill and Crawford[208], by separating crude corn-germ oil into its saponifiable and unsaponifiable constituents, concentrated the antioxidant activity into the latter. This fraction, which corresponded to approximately 2 per cent. of the oil, powerfully stabilised other fats to which it was added. Acetylation of the fraction completely destroyed its activity, but this was largely restored on subsequent hydrolysis (Table 40). It was therefore

concluded that the antioxidants of corn-germ oil are hydroxy compounds. They could not, however, be identified with any of the known sterols present, since these were found to be inactive.

TABLE 40.—*Antioxidant activity of the unsaponifiable fraction of corn-germ oil.*

(Mattill and Crawford[208])

Fat	Unsaponifiable fraction	Induction-period (hours at 70°C.)
(A) 5 g. lard + 10 drops cod-liver oil	—	7·5
,, ,, ,, ,,	10 mg.	10·5
,, ,, ,, ,,	10 mg. acetylated	7·5
,, ,, ,, ,,	10 mg. regenerated	10·0
(B) 5 g. lard + 10 drops cod-liver oil	—	4·5
,, ,, ,, ,,	100 mg.	118·0
,, ,, ,, ,,	100 mg. acetylated	5·0
,, ,, ,, ,,	100 mg. regenerated	73·0

In only a few cases have pure substances possessing antioxidant properties been isolated from fats or fat-containing tissues. From the unsaponifiable fraction of the lipides of lettuce, Olcovitch and Mattill[231] separated a crystalline product to which they assigned the formula $C_{13}H_{14}O_5$. This substance possessed an antioxidant activity (which it lost on acetylation) comparable to that of α-naphthol, a fairly powerful antioxidant, and appeared to contain one or more phenolic groups. Its structure was not further elucidated. Similar methods to those employed for lettuce, i.e., removal of carotinoids and sterols, followed by diphase distribution of the residue between aqueous methyl alcohol and petroleum ether, demonstrated the existence of inhibitors in the tissues of wheat-germ, carrot and tomato. Distribution between solvents and the boiling ranges of the most active fractions indicated that the active substances in all four cases were probably different from one another, and certainly from vitamin E, which was also present[29]. In recent communications Olcott and Mattill[229, 230] have described the preparation of antioxidant-active concentrates from the unsaponifiable fractions of wheat-germ, cottonseed and palm oils. For the active constituents of these concentrates they propose the name "inhibitols," in order to indicate their function as inhibitors, and also the invariable occurrence of hydroxyl groups, upon which their activity is considered to depend. Similar substances are said to be present in lucerne, spinach, corn, sesame, soya-bean and arachis oils, and probably in many other vegetable substances, but are almost entirely absent from yeast, lard, olive, cod-liver, palm-kernel and castor oils.

As obtained from various sources, inhibitol concentrates are light yellow oils of medium viscosity which do not crystallise on long standing, and are stable under ordinary conditions in the laboratory for years. They are destroyed by reagents which attack hydroxyl groups, but in the case of the formation of esters, activity is largely regenerated on hydrolysis. Inhibitols appear to contain at least one double bond, since on reaction with halogens they lose their activity, regaining it on reduction with zinc-dust and acid. They are, however, resistant to hydrogenation, which diminishes, but fails to destroy their unsaturation. All active preparations examined showed a strong absorption-band in the ultra-violet at 2,940 Å which in intensity appeared to be roughly proportional to their activity. Though these substances were probably still far from pure, no further concentration could be obtained by fractional distillation or absorption.

TABLE 41.—*Effect of inhibitols on the oxidation of fats.*
(Olcott and Mattill[230])

Substrate	Inhibitol concentrate added (%)	Days to become rancid at 63° C.	
		Inhibitor	Control
Cottonseed-oil	0·1 cottonseed	3·5, 4	4, 4·5
,, ,, ...	0·05 ,,	8·5	8
,, ,, ...	0·05 wheat-germ	3	3·5
,, ,, ...	0·05 ,, ,,	6·5	8
Hydrogenated cottonseed-oil..	0·02 ,, ,,	27, 31	28, 32
,, ,, ,, ..	0·03 ,, ,,	42, 49	41, 49
Lard	0·02 ,, ,,	9, 9	3, 3
,, ...	0·01 ,, ,,	10·5, 11	4, 4
,, ...	0·02 ,, ,,	50*	10*
Oleic acid	0·03 ,, ,,	10*	4*
Methyl oleate	0·02 ,, ,,	30*	4*

* Induction-period of oxygen-absorption at 75° C. (hours.)

Inhibitol concentrates proved to be effective anti-oxidants for animal fats and for purified (distilled) fatty acids and esters, but they consistently failed to show any antioxygenic activity when added, even in relatively large amounts, to vegetable oils (Table 41). For the investigation of this phenomenon Olcott and Mattill used as substrate crude (undistilled) esters of cottonseed or other vegetable oil, prepared by direct esterification of the fat with methyl alcohol and hydrochloric acid. These crude esters oxidised more rapidly, and with a sharper induction-period, than the original fat, but exhibited a qualitatively similar behaviour towards anti-oxidants. They were not protected by inhibitol concentrates, but were stabilised to a remarkable degree by various organic and inorganic acids (Table 42). The results suggest that in the aliphatic

TABLE 42.—*Inhibiting effect of acidic and phenolic compounds (0·02 per cent.) on the oxidation of the crude esters of hydrogenated cottonseed-oil.*
(Olcott and Mattill[230])

Substance	Antioxidant index	Substance	Antioxidant index	Substance	Antioxidant index
Sulphuric acid (95%) ..	15–20	Arsenic acid	3	Pyruvic acid	10–15
Phosphoric acid (85%) ..	15–20	Oxalic acid	15–20	Hydroquinone	1·2–1·6
Calcium dihydrogen phosphate	4–6	Malonic acid	10–15	Catechol	12
Kephalin	4–6	Tartaric acid	10–15	Pyrogallol	26
Citric acid	10–15	Maleic acid	4–6	α-Naphthol	9
		Malic acid	8–12		

series two carboxyl (or one carboxyl and a carbonyl group) are necessary for activity, and these must not be separated by more than one CH_2 group, unless an active group or groups, such as hydroxyl or a double bond, is also present. The inactivity of the salts and esters of the dicarboxylic acids indicates that the carboxyl groups must be free. Among the inorganic acids tested, sulphuric and phosphoric alone showed marked antioxygenic activity. With the exception of hydroquinone, the phenols were as effective for ester preparations as for lard.

The reactions of the vegetable oils and crude esters thus provide a marked contrast to those of lard, esters of lard, and purified (distilled) fatty acids and esters, in which the acid inhibitors are all inactive or only very slighly active, and the phenols and the inhibitols very efficient. From these data it was concluded that the inhibitol originally present in the vegetable oil, and remaining largely in the residue when the crude esters are distilled, is responsible for the increased effect of the acid type inhibitors in the crude esters. The inhibitors studied were divided tentatively into three groups:—(1) the acid type, (2) inhibitols and hydroquinone, and (3) the phenolic type, including α-napthol, pyrogallol, catechol and others. Vegetable oils and crude esters of vegetable oils are protected by types (1) and (3) but *not* by (2); distilled esters of vegetable oils, lard and esters of lard, and distilled fatty acids and esters are protected by types (2) and (3), but *not* by (1). Numerous data were obtained, showing that in general any type (1) inhibitor, used in conjunction with any type (2) or type (3) compound, prolongs the induction-period of animal fats and of pure esters to a much greater extent than would be expected from a summation of the effects of each used alone. A few of these figures are reproduced in Table 43.

The work so far discussed has been concerned entirely with antioxidants which can be concentrated in the unsaponifiable fractions of fats and oils. Substances insoluble in fat-solvents or decomposed by alkali, e.g., proteins, tannins, glucosides or carbohydrates, may perhaps also assist in protecting fats in the tissue. Some of these will be mentioned again later.

Gossypol, a substance which occurs in cottonseed in quantity up to 0·6 per cent. and in the crude oil up to 0·2 per cent [257], has been isolated in the crystalline form from this source and shown to possess marked antioxidant properties[206, 256]. Gossypol ($C_{30}H_{30}O_8$) contains six hydroxyl groups, some at least of which are phenolic, and two carboxyl. groups[43]. This is an example of a naturally occurring antioxidant which is definitely toxic. Thyroxine, the active principle of the thyroid gland and a phenolic substance, is also an inhibitor of the oxidation of unsaturated fatty acids[69].

Inhibitors of oxidation are known to be present in raw rubber, which on their removal rapidly autoxidises and "perishes." Several of these compounds have been isolated, one being a substance

TABLE 43.—*Effect of mixtures of inhibitors on various substrates.*
(Olcott and Mattill[230])

Substrate	Inhibitor	Induction-period (hours)	
		Inhibitor	Control
Distilled esters of hydrogenated cottonseed-oil	0·04% wheat-germ concentrate	9·5	5
" " " " "	0·1% tartaric acid	8	5
" " " " "	Wheat-germ + tartaric acid	148	5
" " " " "	0·02% wheat-germ concentrate	7·5	5
" " " " "	0·02% citric acid	3·5	3
" " " " "	Wheat-germ + citric acid	98	3
Lard	0·02% wheat-germ concentrate	30	7
"	0·1% malonic acid	5	4
"	Wheat-germ + malonic acid	89	4
Methyl oleate	0·02% wheat-germ concentrate	22	10
" "	Wheat-germ + 0·1% tartaric acid	136	10
Lard	0·02% orcinol	75	12
"	0·1% phosphoric acid	21	12
"	Orcinol + phosphoric acid	>314	12
Fatty acids of lard	0·02% orcinol	29	2·5
" " "	0·1% phosphoric acid	5	2·5
" " "	Orcinol + phosphoric acid	>300	2·5

possessing phenolic properties, and two others ($C_{27}H_{42}O_3$ and $C_{20}H_{30}O$) apparently liquid sterols of unknown structure[36]. Much of the antioxidant activity in natural rubber is, however, probably due to basic (aromatic amino) substances[27]. A different type of inhibitor recently found in rubber latex is of particular interest. Substances of this class, whose chemical structure is unknown, are deep brown in colour and appear to protect the rubber by absorption of actinic light[27, 26]. Their action is not chemical, as shown by the fact that a solution in toluene of a particular substance placed between the source of light and the rubber had the same protective effect as the substance incorporated in the rubber. Other coloured organic compounds such as azo-benzene and α-benzeneazo-β-naphthylamine (but not carotene) also prevented oxidation when used in solution in toluene as a light-filter and, if soluble, when incorporated in the rubber. Comparison of the absorption-spectra of effective substances showed that the wavelengths of light which promote the autoxidation of rubber lie between 3,000 and 4,800 Å, a range which agrees well with that found effective in case of fats (page 151). Pigments present in natural fats and fatty tissues may perhaps, in a similar way, protect them from sunlight.

Substances which contribute to the stability of fats may thus be of many types, and research into their nature and properties has hardly yet begun. It is to be hoped that use of the more recently devised methods of organic chemistry, such as that of fractional adsorption on active surfaces, may soon lead to more rapid advances in knowledge.

Stabilisation by the Use of Natural Antioxidants

Since it was first shown that corn and wheat-germ oils[5, 251], or their unsaponifiable fractions[208], could be used to improve the resistance to oxidation of less stable fats, various means of utilising the natural inhibitors present in crude oils and in vegetable tissues have been suggested. Patents have been taken out, for example, to cover the addition of 5–10 per cent. of sesame-oil (hydrogenated to a suitable consistency) to lard, beef-fat and shortening fats[114], and of crude cottonseed-oil to refined cottonseed-oil or to lard[117].

Musher's patents[221] involve the application of a somewhat similar principle. In this case the fat or foodstuff is treated with a finely divided vegetable material such as oat, barley or soya-bean flour, crushed sesame-seed, peanuts, linseed-cake, soya-bean press-cake, castor-bean pomace, corn-germ cake, corn-gluten or, in the case of roasted coffee, with a small proportion of finely ground unroasted coffee. The flours of the cereals are recommended for vegetable oils and for the majority of general purposes, oat-flour being most suitable on account of its mild flavour and absence of colour. Wheat-flour is said to be low in activity. The finely ground or crushed oil-bearing seeds (soya, sesame and peanut) are apparently more suitable for use with animal fats, though crushed sesame-

seed, which has a sweet, nutty flavour, may be used for biscuits and pastry. Soya-bean flour for use with lard may be bleached and extracted with a solvent in order to avoid interference with the natural flavour, but the loss of a considerable proportion of its activity occurs in the process. Alternatively, the untreated flour

TABLE 44.—*The effect of cereal-flours and of crushed oil-seeds on the oxidation of lard.*

(Musher[220])

Substance added	Induction-period (hours at 98° C.)	
	Prime steam lard	Bleached lard
—	5	1·5
Raw soya-bean flour 1%	—	2·7
„ „ „ 2%	—	5·2
„ „ „ 5%	35	16·2
„ „ „ 10%	65	47·7
Raw soya-flour 5% and filtered*	—	9·0
„ „ 10% „ „	—	14·7
Bleached and extracted soya-flour 10%	9	—
Bleached crushed sesame-seed 5%	31	—
„ „ „ „ 10%	49	—
Sesame seed 5% and filtered*	—	7·5
„ „ 10% „ „	—	13·0
Oat flour 1%	—	2·5
„ 2%	—	3·2
„ 5%	16	5·5
„ 10%	22	9·0
Oat flour 5% and filtered*	—	3·0
„ 10% „ „	—	6·2

* After holding the mixture at 65° C. for 30 minutes. The other samples were not filtered.

may be added to the melted lard and subsequently filtered out, or the flour contained in a bag of filter-cloth may be immersed in the fat. Table 44, which shows the effects of these treatments on the susceptibility of lard, is compiled from data obtained by the aeration-method at 98° C.[220]

Oat-flour may be incorporated directly into lard or shortenings, and the mixture used for baking purposes. Other foods which it is suggested might be treated with oat-flour include (*a*) by direct addition, margarine, ice-cream, mayonnaise and salad-dressings; (*b*) by sprinkling or dusting the exposed surface, bacon, potato-chips, salted nuts, shredded suet and fatty fish[220]. A further proposed application of this principle is by sizing parchment-paper with oat-flour, or by incorporating the flour in the paraffin wax used for the preparation of waxed papers. It is claimed that the use of such papers for the packing of fatty foods retards the development

of rancidity, which usually appears first in the film of fat on the wrapper. Further data on the use of cereal-flours for the treatment of various foods have recently been published[233, 203].

The protection obtained in all of these cases is attributable to the diffusion of a portion of the natural antioxidant from the vegetable tissue into the fat. The number of foodstuffs which can be treated with the relatively large proportion of the flours required is limited. It is possible, however, that preservation by means of the natural antioxidants of vegetable tissues may be made of wider application by the use of much smaller quantities of potent extracts, prepared by the treatment of cereal-flours or of oil-seed press-cakes with suitable solvents, *e.g.*, alcohol.

In this connection recent work by Green and Hilditch[104] is of interest. These investigators treated freshly extracted soya-bean meal by digestion with 2 per cent. acetic acid in water or acetone, followed by extraction with methyl alcohol. In this way a product was obtained which, on extraction with acetone, gave about 2 per cent. (on the original meal) of a viscous gum which possessed marked antioxidant properties. At a concentration of 0·02 per cent. the soya-bean extract increased the induction-period of distilled olive-oil esters at 97·5°C. by from four to eight times. It is significant that the yield of inhibitor (still probably very impure) was much greater from the extracted oil-cake than from the fatty oil itself, indicating that the oil removes from the seed only about 2 or 3 per cent. of the total amount of inhibitor present. This work suggests that edible fats might be stabilised by the incorporation of concentrates of natural inhibitors recovered from extracted seed-cakes. Further information, however, is required concerning the chemical nature of the inhibiting substances themselves.

Other Inhibitors

The empirical use of various substances for the stabilisation of fats dates back many years. Deschamps' discovery (1843) that gum benzoin improves the keeping properties of lard has since been widely utilised in the preservation of this fat for pharmaceutical purposes. Chevreul (1856) was familiar with the fact that oak and certain other woods delay the drying of linseed-oil, and American Indians are said to have used the bark of trees for retarding the development of rancidity in bear-grease. These are both somewhat indirect methods of incorporating phenolic antioxidants in the fat.

The work of Moureau and Dufraisse showed that many substances are capable of retarding the atmospheric oxidation of fats, certain phenols and organic nitrogenous substances being particularly effective. Much of the subsequent work on the stabilisation of fats has been carried out with substances of these types.

Phenols.—Attempts at the correlation of antioxidant properties with molecular structure have led, in the case of the phenols to the elucidation of certain basic principles which appear to determine activity. Moureau and Dufraisse[218], in their original studies on the

autoxidation of acrolein, showed that catechol, hydroquinone (quinol) and pyrogallol were much more powerful antioxidants than phenol and resorcinol, and these conclusions have since been confirmed and extended. Tanaka and Nakamura[286, 222] compared the changes in iodine-value, refractive index, viscosity, acid value and specific gravity of various oils containing inhibitors on exposure to sunlight. Their results placed the phenols in descending order of efficiency as follows:—pyrogallol> hydroquinone> catechol> eugenol> thymol> α-naphthol> β-naphthol, phloroglucinol, resorcinol and phenol. The cresols (o> p> m) and mononitrophenols were more active than phenol. Wagner and Brier[307] assessed the relative efficiencies of several phenols in prolonging the induction-period of linseed-oil at 100°C. as hydroquinone 100, pyrogallol 70, α-naphthol 40, resorcinol 4 and β-naphthol 0.

Mattill[206], measuring the induction-period (absorption of oxygen at 70°C.) of a mixture of lard and cod-liver oil in the presence of 0·02 per cent. of inhibitor, expressed his results in terms of protection factors as follows:—

Hydroquinone	120*	Benzoquinone	9	o– and p– Cresols	1–2
Catechol	>55	5–Methylresorcinol	4	Thymol	1–2
Pyrogallol	>55	Gossypol	4	Catechol mono-methyl ether	1–2
α–Naphthol	31	Phloroglucinol	3	Resorcinol	1–2
β–Naphthoquinone	25	β–Naphthol	1–2		

Later determinations [227] by a similar method, but using 0·01 per cent. of the substance in lard at 75° C gave:—

Pyrogallol	>60	α–Naphthol	22	Methylhydroquinone	7
Hydroxyhydro-quinone (1, 2, 4)	60	Dihydroxynaph-thalene (1, 8)	20	Benzoquinone	4
Catechol	41	Tetrahydroxyben-zene (1, 2, 3, 4)	20	Toluquinone	2
Hydroquinone	38	β–Naphthoquinone	8	Pyrogallol mono-carbonate	2
				Thymohydroquinone	2

Greenbank and Holm[111], comparing the peroxide-contents of treated (0·01 per cent.) and untreated samples of fats after storage at 42°C. for 10 days, obtained protection-factors for hydroquinone 3·7, hydroxyhydroquinone and pyrogallol 3·6, catechol 2·0, resorcinol 1·1, and phloroglucinol and phenol 1·0.

General conclusions arising from these results may be summarised as follows. Monohydroxybenzene (phenol) is practically inert, but the substitution of suitable groups increases its activity. Thus the o- and p-cresols and nitrophenols, thymol (2-isopropyl-5-methyl-phenol) and eugenol are all active. The introduction of a second hydroxyl group in the ortho (catechol) or para (hydroquinone) position enormously increases activity, while the substitution of a third hydroxyl to form pyrogallol (1, 2, 3) or hydroxyhydroquinone (1, 2, 4) still further enhances the effect. Activity now appears to have reached a maximum, tetrahydroxybenzene (1, 2, 3, 4) being

* The extremely large protection factors obtained by Mattill and his co-workers may be due to their using, for testing purposes, slightly oxidised fats of short induction-period.

only half as powerful as the 1, 2 and 1, 4 diphenols, and hexahydroxybenzene a completely inactive substance. The meta compounds resorcinol (1, 3) and phloroglucinol (1, 3, 5) are only very feeble inhibitors.

It appears to be necessary for antioxidant activity that the hydroxyl groups be directly substituted in an aromatic nucleus. Hydroxylated hexamethylene rings, for example, are inert, as are substances such as phenol-o- and p-carboxylic acids, and o-hydroxybenzyl alcohol, in which the second hydroxyl group is in a side chain. The hydrogen atoms of the phenolic groups must also be free. Thus, esterification of one or both of the hydroxyl groups of hydroquinone destroys its activity, while alkylation of one group greatly reduces, and of both completely destroys activity. Of the naphthols, the α-compound is a powerful antioxidant, the β- weak. 1, 8-dihydroxynaphthalene is fairly active, the 1, 3 compound less so and the 1, 4 inactive. A number of more complex phenolic substances have been patented for use as antioxidants in fats and soaps, *e.g.*, p-hydroxydiphenyl[154], p-hydroxy-diphenylmethane, and p-hydroxydiphenylether[37].

These data should prove of considerable value in the identification of antioxidant-active substances of natural origin, some of which have already been shown to contain only carbon, hydrogen and oxygen[207], while a few are definitely known to be phenolic in nature.

Amines and Other Antioxidants.—The second main group of substances which function as inhibitors of the oxidation of fats comprises the aromatic amino compounds. Aniline, like phenol, is only a feeble antioxidant, but a number of the substituted amines, particularly secondary compounds such as phenyl-α-naphthylamine and diphenyl-p-phenylene-diamine, are very active, as are certain amine-aldehyde condensation products (aldol-α-naphthylamine) and amino-phenols, diphenylhydrazine and diphenylguanidine. Substances of these general types are used in a wide variety and on a very large scale for the stabilisation of rubber. A list giving the chemical constitution and commercial names of a number of these rubber antioxidants has been published by Jacobs[158]. It is largely owing to the use of such substances that modern motor-tyres outlast their predecessors of 20 or 30 years ago by as much as five to ten times. A number of the antioxidants used for rubber, *e.g.*, acetaldehyde-ethylenediame, diphenylethylenediamine, phenyl-α-naphthylamine, and phenyl-β-naphthylamine have been shown to be effective inhibitors of the oxidation of fats,[303, 286] but cannot, of course, be used in edible products. Sugar amines have, however, been suggested for this purpose[150].

Substances of various types such as cyanamide compounds[120], aminosulphonic acids, aliphatic alcohols obtained by catalytic hydrogenation of fat[75], sodium sulphite, hydrosulphite and thiosulphate[121], stannous chloride, formaldehyde and hexamethylenetetramine[272], have been proposed as antioxidants, particularly

for use in soaps in which the reactive polyphenols and amines are much less effective than in dry oils.

Effect of Water on the Activity of Antioxidants.—Several investigators have pointed out that substances such as the simple polyhydric phenols and amines, which are powerful antioxidants for dry fats, lose their activity when the fat is treated with water, particularly at a slightly alkaline *p*H, or when mixed and heated with any material containing moisture and protein[224, 113]. Thus, the addition of 0·05–0·1 per cent. of pyrogallol to fresh lard will preserve it almost indefinitely under ordinary conditions of storage, but the stabilized fat when made up into pastry or biscuits will not remain sweet for an appreciably longer period than the untreated lard. Substances of these types may also cause discoloration, particularly in the presence of weak alkali.

Attempts have recently been made to overcome these difficulties by the use of substances insoluble in water but soluble in fat, such as can be obtained by the substitution of the simple polyhydric phenols or amino phenols. Examples claimed in patents are the pyrogallol-stearic acid compound $C_6H_2(OH)_3.CO.C_{17}H_{35}$, pyrogallol-dimethyl ether $OH.C_6H_3(OMe)_2$, the 1, 4 and 1, 5 dihydroxynaphthalenes $C_{10}H_6(OH)_2$, and the product obtained by the condensation of acetone with pyrogallol[246, 247]. Such substances are said to be odourless, tasteless, non-toxic and effective in the presence of water, but no data have been published concerning their activity or toxicity.

Non-toxic Antioxidants

Substances such as the polyphenols and amines described above are undoubtedly toxic when ingested in quantity, though it is doubtful whether in the small amounts required they would be any more harmful than some of the natural constituents of foods. Hydroquinone, for example, at a concentration sufficiently high to stabilise the fat and fat-soluble vitamins in experimental diets, has been fed to rats for a number of generations without any apparent ill effects[231]. Nevertheless, in the absence of more definite evidence of non-toxicity such substances cannot be added to foods. They have, however, found limited application in fats employed for other than food purposes.

During the last few years increasing numbers of attempts have been made to utilise as antioxidants substances of undoubtedly non-toxic character. Of these carotene and lecithin, which occur naturally in association with fats, are of particular interest, but the evidence regarding their effect on oxidation has been very conflicting.

Carotene.—Some investigators have found that carotene accelerates oxidation,[231, 91, 111] others that it functions as an antioxidant[211, 224]. Oxidised carotene has been said to be a prooxidant[211]. Newton[224] observed that hydrogenation, which bleaches the pigment, increased rather than reduced its protective

properties, but the material employed in this case (palm-oil or an extract of carotene-rich vegetable substances such as carrots or lucerne) would undoubtedly contain other inhibitors of oxidation which are known to accompany the carotinoids in plants (page 163).

A probable explanation of these conflicting results has been advanced by Bradway and Mattill in a recent paper[29]. These authors state that crude carotene, as separated from plant sources, usually shows protective properties owing to the association with it of traces of contaminating substances which are powerful inhibitors. Purified carotene (or lycopene) from any source slightly *reduces* the induction-period of fats, and is said in addition to increase the rate of subsequent oxidation[92]. The observation that carotene inhibits the absorption of oxygen by rapidly oxidising linoleic acid or linseed-oil[211] may, however, be an instance of the phenomenon of inversion referred to on page 181.

Lecithin.—Similar results have been obtained in the case of lecithin. Antioxidant protection factors of 1·5–3·0[254] and 1·7–1·8[171] were reported for 0·1–0·2 per cent., while other observers [251, 299] failed to obtain any effect by the addition of relatively large amounts of this substance. Experiments in which vegetable lecithin and cyanide were found to be more active even than hydroquinone and diphenylguanidine[85] were carried out in the presence of a cobalt salt, and cannot be considered to afford evidence of activity in the absence of the metal.

These conflicting data have in some degree been reconciled by the recent work of Olcott and Mattill[228], who found that crude lecithins of brain, egg, and soya-bean slightly inhibited oxidation, as did several samples of commercial lecithin. Protection factors obtained were 1·3–1·6 for 0·2 per cent. in lard, and 2–4 for 0·1 per cent. in cottonseed-oil. No separation of active substance could be obtained by fractional precipitation of these products with acetone, but egg-lecithin purified *via* the cadmium chloride compound was completely inactive. Purified kephalin, on the other hand, possessed fairly marked protective properties and produced its maximal effect at low concentrations. It was therefore concluded that the active constituent of commercial " lecithins " is actually kephalin, pure lecithin being inactive.

Maleic Acid.—Greenbank [106], in attempting to isolate natural antioxidants from cottonseed and from soya-bean meal, observed that certain water-soluble fractions which possessed protective properties were slightly acidic. He therefore investigated the effect of the addition of organic acids of known constitution on the rate of oxidation of a number of fats. Maleic acid at a concentration of 0·01 per cent. was found to increase the length of time necessary for the development of rancidity by a factor of approximately 3. Other acids showed protection in lesser degree[111], citraconic, itaconic and aconitic acids (homologues of maleic) giving factors of 2·0, 1·9 and 1·6 respectively, and citric 1·8, fumaric and malic 1·3 and tartaric

1·2. The activity of these acids was said to be greatly reduced or completely destroyed[105] by the presence of water. Salts and esters of the acids were also found to be inactive. Patents have been taken out to cover the use of maleic acid[107], which is claimed to retard the development of rancidity in dried milk, pastry and caramels[72]. Several subsequent investigators, however, have failed to obtain any protective effect from the use of this substance in dry fats[299 227], and it is only feebly active in a fat-water system[189]. The explanation of some of these conflicting results may lie in the different response to acidic inhibitors shown by fats of different types (page 167).

The toxicity of maleic acid is said to be of the same order as that of oxalic acid[72] which, though poisonous in quantity, is consumed in appreciable amounts in common vegetables.

Gum Guaiac.—Arising from the use of gum benzoin for the stabilisation of pharmaceutical ointments containing lard, Grettie[113] examined a number of other natural gums and resins for activity as inhibitors of oxidation. Of these gum guaiac was the most effective and could be incorporated in fats in adequate quantity without imparting an unpleasant flavour. The most notable property claimed for this substance is the power of remaining active in the presence of water, and in baked foods. Table 45, which summarises the more important data presented, indicates that gum guaiac is an efficient antioxidant for lard. It is stated to be much less effective

TABLE 45.—*The effect of gum guaiac as an antioxidant in baked foods.* (Grettie[113])

Sample	Days to become rancid with		
	0%	0·05%	0·1%*
Lard at 70° C.	5	12	15
,, 35° C.	94	—	>538
Pastry at 70° C.	2	4	14
,, 35° C.	9	65	—
,, ,, ...	17	—	360
Crackers at 70° C. ..	3	—	9
,, ,, 35° C. ..	37	—	>200

* Percentage of inhibitor incorporated in the lard shortening used.

for more highly unsaturated fats, such as cottonseed-oil. No definite information is available concerning the toxicity of this substance, but it has been used to some extent as a constituent of medicines without any apparent ill effects.

Water-soluble Inhibitors in a Fat-water System.—In the greater part of the published work on pro-and anti-oxidants the protective effect produced by the addition of the substance to the pure, dry fat has been measured. Further, in order to obtain results quickly,

the determination has usually been carried out at some fairly high temperature (70°-100° C.), or by use of light or a metallic salt as an accelerator. A procedure of this kind is open to several objections.

In the first place, the majority of foods contain fat in contact with an aqueous phase, and it has already been pointed out that inhibitors which are effective in the dry fat are not necessarily so under these more complex conditions. Further, the use of a metallic accelerator seems likely to lead to erroneous results, in that substances

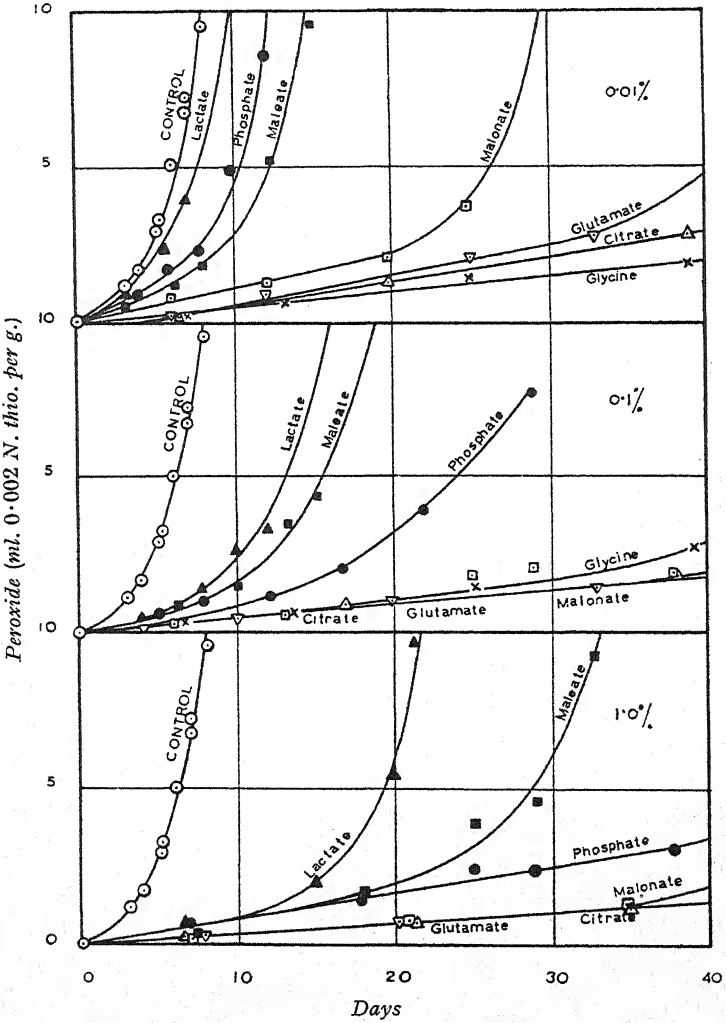


FIG. 29.—*The autoxidation of lard at* 20°C. *in contact with an aqueous phase at pH* 7·0 *containing various non-toxic antioxidants.* (Lea[189])

which inhibit the pro-oxidant effect of the metal, but have no effect on the oxidation of the fat, will be classed as antioxidants. The case of lecithin has already been mentioned (page 175), and other experiments[189] indicate that cyanide, which appears to be a powerful antioxidant in the presence of traces of copper, has no protective action in its absence. The use of high temperature and of light is less open to objection, but even here the stability of different components of the system—peroxides, the inhibitor itself, etc.—may be appreciably altered, with consequent risk of error in applying the results obtained to ordinary conditions of storage. A method of this kind must, however, be used when results are required quickly, and experience has shown that data thus obtained usually furnish a useful guide to behaviour during storage.

TABLE 46.—*The effect of water-soluble antioxidants on the oxidation of lard at* 20°C.

(Lea[189])

Solution		Induction-period*	Protection-factor
Borate buffer at *p*H 7·0		5·6	1·0
Buffer + diethanolamine ..	0·01%	7·1	1·3
,, + ,, ..	0·1%	14·0	2·5
Buffer + phosphate	0·1%	16·7	3·0
Buffer + malonate	0·1%	16·8	3·0
Buffer + citrate	0·01%	23·8	4·3
Buffer + phosphite†	0·1%	25·0	4·5
Buffer + pyrophosphate ..	0·1%	ca. 31	ca. 5·5
Buffer + citrate	0·1%		
Buffer + glycine	0·01%		

* Days to reach 6 ml. per g.
† Contains also 0·44 per cent. phosphate.

In some recent experiments (Fig. 29, Table 46) Lea has investigated the effect of a number of water-soluble substances on the rate of oxidation of lard in contact with an aqueous phase at 20° C. General conclusions were as follows :—

(1) Aliphatic polyhydroxy compounds, such as glycerol and the sugars, are weak antioxidants. Glycerol at a concentration of 0·25–0·5 per cent. has previously been shown to retard the development of rancidity in cereal products[89] and in fat[251]. The mono- and diglycerides which are coming into use for improving the emulsifying properties of edible fats may therefore have some beneficial effect on keeping properties.

(2) Aliphatic monobasic hydroxy-acids (e.g., lactic and glycollic), maleic acid and the ethanolamines, are moderate antioxidants. Polybasic hydroxy acids (e.g., tartaric and citric) and malonic acid are powerful antioxidants.

(3) Aliphatic amino acids, e.g., glycine, aspartic and glutamic acids, and asparagine are all powerful antioxidants. Protein has

also considerable antioxidant activity, and may therefore assist in the stabilisation of crude oils and of fats in the tissue.

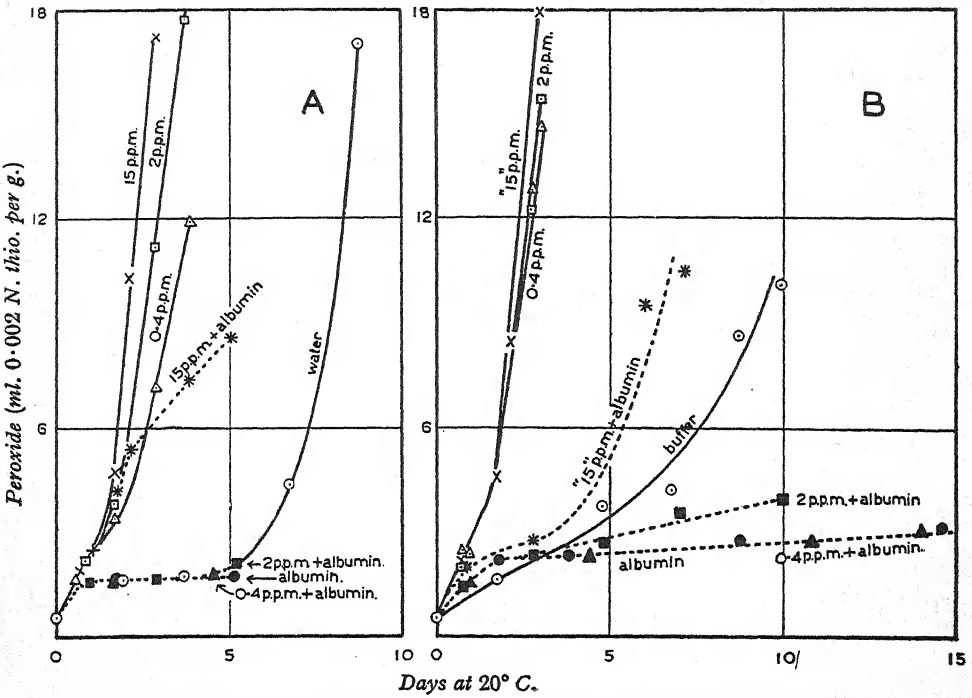


FIG. 30.—*The pro-oxidant effect of copper in the presence of protein* (4% *albumin*) *in* (A) *water* and* (B) *borate buffer at pH* 7·0. (Lea[189])

(4) The pro-oxidant effect of copper at low concentrations (ca. one part per million under the experimental conditions employed) is completely inhibited by protein, cyanide and other antioxidants. At higher concentrations of the metal even 4 per cent. of protein fails to prevent a powerful acceleration of oxidation (Fig. 30).

(5) These water-soluble antioxidants still inhibit oxidation when the water-content of the fat is as low as 0·25 per cent.

(6) They are active at *p*H 7, as well as in more acid solutions.

(7) The more powerful of the substances examined were effective at concentrations as low as 0·01 per cent., producing an extension of the induction-period of from two to six times. The magnitude of the factor obtained varied with the sample of fat, and in the presence of traces of copper was sometimes as high as 20. Two of the substances tested at a concentration of 0·02 per cent., failed to produce any additional protective effect when added to a system

* Solutions containing protein could not be kept for longer than 5 days owing to the growth of micro-organisms.

already containing 4 per cent. of albumin. It seems therefore that the usefulness of water-soluble antioxidants of this type may be limited to cases in which no considerable amount of soluble protein is present.

Phosphoric Acid.—Several American patents [73, 248] cover the use of ortho- and meta-phosphoric acids and their salts having an acid reaction, as inhibitors for direct addition to oils and fats. Lea, [194, 189] working with a fat-water system, found pyrophosphoric considerably more effective than the ortho acid, and both were certainly active in neutral solution. Phosphite is also a useful antioxidant, and possesses the advantage of being a reducing agent which on oxidation yields phosphoric acid, itself an antioxidant. The use of acid phosphites for the prevention of rancidity in cereal products, flours, etc., has been patented[54].

Mechanism of the Action of Antioxidants

As already pointed out (page 137), a substance which inhibits the autoxidation of fats does so by breaking the reaction-chains, and as a result is usually oxidised itself. The inhibitor is thus gradually decomposed during the induction-period and finally disappears with its conclusion, a fact which has been experimentally verified in a number of cases[30, 92].

Substances which prolong the induction-period of a fresh fat also arrest the absorption of oxygen and formation of peroxides for a limited period after rapid oxidation has commenced[272]. Efficiency in this respect, however, decreases rapidly[92] as oxidation proceeds, owing to the accumulation in the fat of reactive peroxides which destroy the inhibitor (page 152). A precisely similar behaviour has been observed in the autoxidation of benzaldehyde. In this case the addition of 0·0002 M. anthracene before admission of the oxygen delayed oxidation for 200 minutes. Added 11 minutes after commencement of the reaction, the delay produced was only 19 minutes (Fig. 28). Ordinary fat peroxides have also been shown to be capable of destroying phenolic inhibitors, but in this case the reaction is slow[319].

Finally, many of the substances which function as antioxidants, themselves autoxidise in a similar manner to the oils[319], and this oxidation is greatly accelerated by traces of active metals[320].

Effect of Concentration

Moureau and Dufraisse in their earlier papers [219] found that, in general, the retardation of oxidation by an inhibitor varied with the amount added, but that the actual relation between concentration and activity was not the same for all substances. On theoretical grounds it might be anticipated that if the antioxidant is destroyed only in the process of breaking reaction-chains, the induction-period should be prolonged by a time directly proportional to the amount of antioxidant added. This is sometimes found to be true over a limited range of concentrations[307, 320, 92], as shown for

hydroquinone and for orcinol in Fig. 31. Thermal decomposition or volatilisation of the antioxidant, decomposition in side reactions with peroxide or other products of the oxidation of the fat, or pro- or anti-oxidant activity of products of the decomposition of the inhibitor may, however, produce concentration/activity curves of the types shown in Fig. 31. The protection afforded frequently approaches a maximum with increasing concentration. After the conclusion of the induction-period the rate of oxidation is usually found to be identical with that of the uninhibited reaction, [272, 320, 307] but may be slower, as in case of diphenylamine[320] where products of secondary reactions are probably responsible.

Non-reducing Inhibitors

The earlier workers in this field were of the opinion that an antioxidant must also be a reducing agent or, at least, a readily oxidisable substance[217], and in the majority of cases this relationship undoubtedly exists. A considerable number of exceptions, however, has been discovered. Anthracene inhibits the autoxidation of anethol, and though not itself susceptible to attack by oxygen, is oxidised to anthraquinone in doing so. Anthraquinone, on the other hand, is a much more powerful inhibitor of this autoxidation, and yet can be recovered unchanged from the oxidised anethol[210]. Phosphoric acid, as already mentioned, is another instance of an inhibitor which is not readily oxidisable. In such cases it is possible that the molecules of inhibitor break the reaction-chain by forming a loose addition compound with the active molecules of the autoxidising substance.

Inversion of Activity

Nakamura[222] has recently observed inversion of the sign of the effect produced by certain substances on the oxidation of oils exposed to sunlight. Several feebly active compounds such as *p*-nitroaniline, vanillin and *β*-naphthol first accelerated and then retarded oxidation, inversion occurring at a point which depended on the iodine-value (i.e., rate of oxidation) of the oil, and on the concentration of the added substance. Powerful pro- and anti-oxidants showed no inversion.

The Influence of Water on Oxidative Rancidity

Early writers generally attributed to water the property of accelerating the production of " rancidity " in fats, and some even considered its presence essential. Later work has favoured the view that moisture, though increasing enzymic and microbial spoilage, may under certain circumstances retard oxidative deterioration. Thus, Holm, Wright and Greenbank[144] observed that rancidity, as judged by odour and taste, appeared much more rapidly in very dry samples of milk-powder than in those containing 2-3 per cent. of water, and suggested that water favoured the further oxidation of aldehydes produced during autoxidation, to less odorous acids[109]. Somewhat similar results have been obtained with powdered

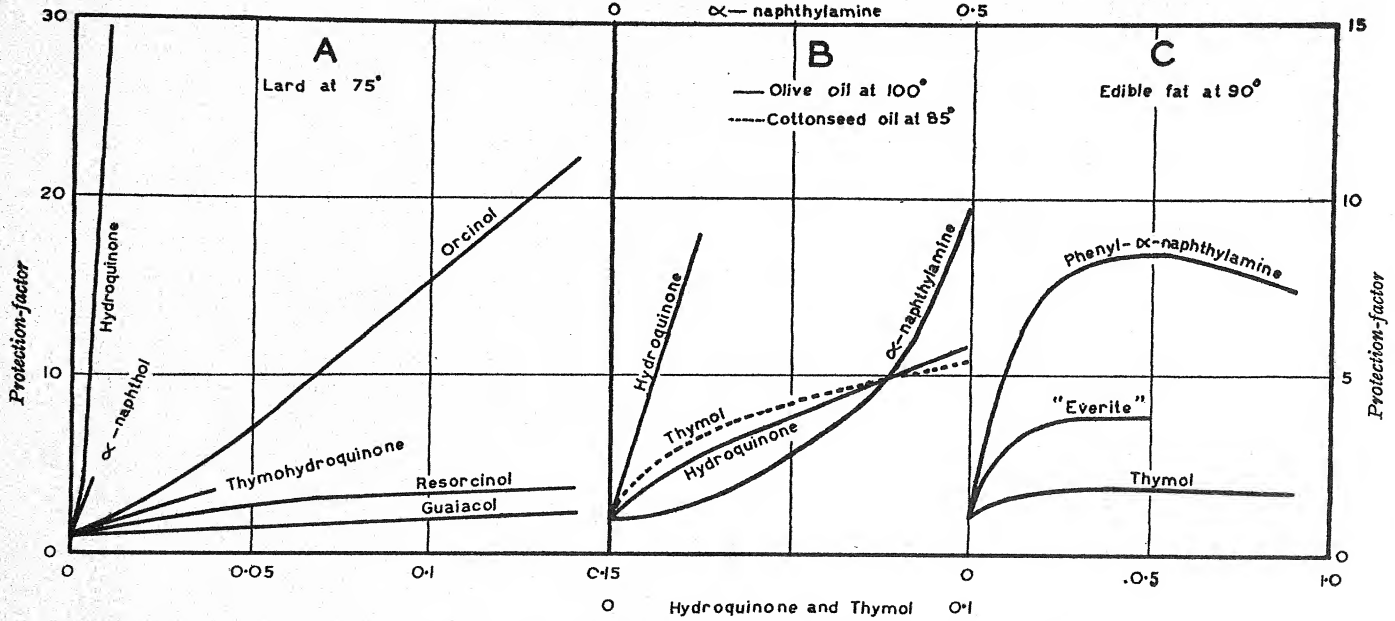


FIG. 31.—*The effect of the concentration of the inhibitor on the absorption of oxygen by fats**

* Calculated from data by (A) French, Olcott and Mattill,[82] (B) Yamguchi[350] and Smith and Wood,[272] (C) Vibrans[203].

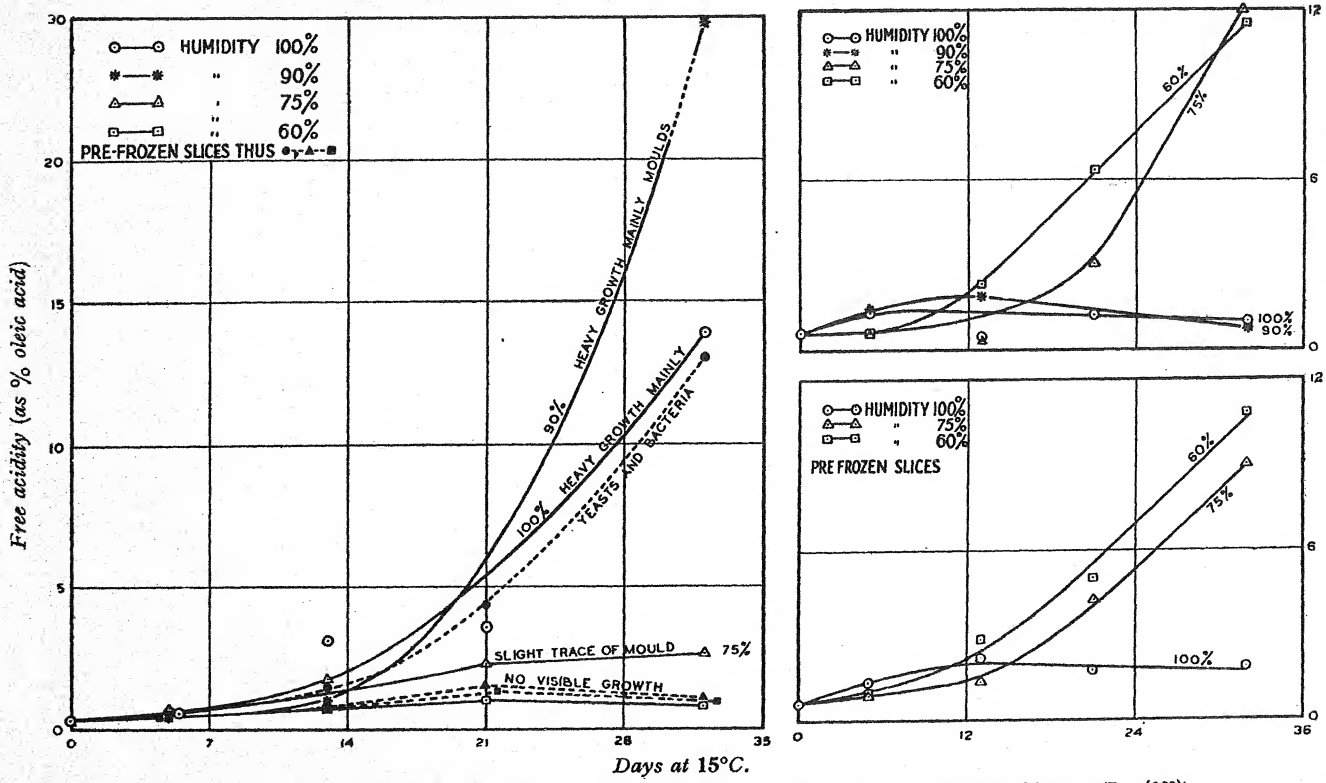


FIG. 32.—*The effect of atmospheric humidity on hydrolysis and oxidation in the fat of bacon.* (Lea[183])

crackers containing 5–6 per cent. of moisture, samples stored over concentrated sulphuric acid becoming rancid more rapidly than controls at atmospheric humidity[298]. In the case of cereal-flours, tallowy odours have been found to develop rapidly in samples containing less than 2 per cent. of moisture, while 5 per cent. gave considerable, and 10–12 per cent. very marked protection from this form of spoilage[89]. Addition of water, followed by further storage, even tended to be curative of tallowiness in dry flours. Similarly, in the case of mixtures of cod-liver oil and milk-powder, 10 per cent. of water greatly delayed the appearance of tallowiness, though mustiness and discolouration developed[5].

All of these data refer to the influence of moisture in systems containing constituents other than fat. In such a case it is possible for the water to achieve its effect indirectly by increasing the antioxidant action of water-soluble substances such as protein, or when present in quantity, by favouring the growth of micro-organisms. Proliferation of a mixed flora on a fatty substrate frequently produces conditions unfavourable for oxidation of the fat. Fig. 32 shows the changes in free acidity and peroxide values in the fat of bacon stored in still air at various humidities. In the moist atmospheres (90 and 100 per cent.) micro-organisms grew rapidly and oxidation was inhibited. At low humidities growth was retarded and the fat oxidised.

The influence of moisture on the rate of oxidation of a pure fat is open to some doubt. Greenbank and Holm[109] found that water increased the length of the induction-period (absorption of oxygen) of butter-fat at 95° C., but more recent investigation failed to detect any influence on the induction-period of lard at 50° C.[92]. In experiments at room temperature, water frequently shortened the induction-period of lard in glass[189], but this effect was probably due rather to solution of pro-oxidants from the glass than to any direct action. Films of lard suspended over water have been found to oxidise appreciably less rapidly than similar films suspended over concentrated sulphuric acid[198].

The most reasonable conclusion to be drawn from the data at present available is that water probably has a slight retarding effect on the rate of absorption of oxygen by fats. It can also influence the rate of oxidation by serving as a vehicle for accelerators or inhibitors, or by permitting the growth of micro-organisms which in turn affect the oxidation. The absence of moisture may perhaps favour the accumulation of the more odorous products of rancidity.

SECTION 7.—THE STABILISATION OF FATS BY HYDROGENATION

In the immediately preceding pages the external factors of temperature, light, and the presence of chemical accelerators and inhibitors have been examined, and shown to be capable of controlling the rate of oxidative deterioration of fats over a very wide

range. The chemical structure of the fat itself, nevertheless, remains a factor of considerable importance.

It has already been pointed out (page 86) that, in general, the more highly unsaturated a fatty acid or glyceride, the more rapidly will it tend to oxidise on exposure to the air. Coconut-oil, for example, which has an iodine-value of 9 and contains practically no acids with more than one double-bond, is enormously more resistant to atmospheric oxidation than a fish-oil of iodine-value 200, which contains a high proportion of acids with from two to six double bonds in the molecule.

Since the first industrial application (1902) of Sabatier's discovery that liquid, unsaturated fatty acids can be converted into the corresponding solid, saturated acids by treatment with hydrogen in the presence of a nickel catalyst, the practice of hydrogenating fats has attained the magnitude of a large industry. Enormous quantities of whale- and fish-oils, cottonseed, soya-bean, arachis and other vegetable oils are annually converted into solid fats, in which form they find use in margarine, cooking fats, pastry shortenings, soaps and candles. Naturally, in destroying the highly unsaturated acids of the liquid oils, stability towards oxidation is greatly increased, particularly as part at least of the natural anti-oxidant of the original oil seems to survive the treatment. A vegetable

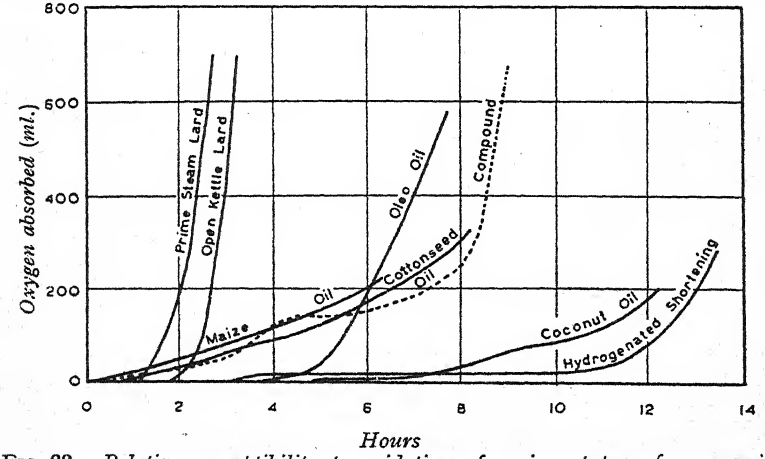


FIG. 33.—*Relative susceptibility to oxidation of various types of commercial shortenings. 200 g. samples stirred in oxygen at* 95° *C.* (Triebold and Bailey[298])

shortening prepared by partial hydrogenation of cottonseed-oil possesses fairly similar physical properties to lard, but is considerably more resistant to oxidation than either the animal fat or the original vegetable oil (Fig. 33).

Slight Hydrogenation

By slight hydrogenation it is possible to increase the stability of solid fats without greatly changing their physical properties.

Lard, butter-fat, palm-oil and cacao-butter, for example, consist mainly of glycerides of saturated and of oleic acids, but contain in addition relatively small proportions of linoleic, and even traces of more highly unsaturated acids. Oleic, almost equally with the highly unsaturated acids, tends to produce a liquid fat, but is much less susceptible to oxidation. It follows therefore that if acids containing two or more double bonds can be made to combine with sufficient hydrogen to bring about their conversion to oleic acid, the physical characteristics of the fat will not be greatly altered, but its keeping properties will be considerably improved.

TABLE 47.—*Changes in the linoleic-acid content of lard with progressive hydrogenation and oxidation.*

(Zeleney and Bailey[324])

	Iodine-value of fatty acids	Thiocyanogen-value of fatty acids	Acids with 1 double bond* (%)	Linoleic acid (%)	Total unsaturated acids (%)	Total saturated acids† (%)
Hydrogenated						
—	65·4	54·9	49·1	11·8	60·9	39·1
—	61·4	54·7	53·1	7·6	60·7	39·3
—	56·9	54·2	57·0	3·1	60·1	39·9
—	51·0	50·0	54·4	1·2	55·6	44·4
Oxidised (hours)						
0	71·5	60·9	55·9	11·8	67·7	32·3
4	69·8	60·8	57·6	10·0	67·6	32·4
7	64·0	58·6	59·1	6·0	65·1	34·9
15	52·0	51·2	56·0	0·9	56·9	43·1

* Includes linoleic acid with one of its double bonds oxidised.
† Includes unsaturated fatty acids with all double bonds oxidised.

Only an approximation to this ideal can be obtained in practice. A mixture of esters or glycerides of oleic and linoleic acids can be hydrogenated so that a large proportion of the linoleic is converted to monoethylenic acid before any considerable amount of stearic acid is produced. The monoethylenic acid, however, consists of a mixture of ordinary, liquid oleic acid with solid (*iso*oleic) isomers. The data given for lard in Table 47 show that slight hydrogenation which reduced the total unsaturated (liquid) acids by only 1·3 per cent., destroyed 74 per cent. of the linoleic acid originally present, while a decrease of 8·7 per cent. in the total unsaturated acids reduced the linoleic acid by 90 per cent. The greater susceptibility of linoleic acid to oxidation is shown by the relatively high rate at which it disappeared when the fat was heated in air.

Slight hydrogenation is useful in improving the quality of low-grade lard, and of oily lard produced by animals raised on softening feeds. Animal fats of low grade tend to possess an unpleasant flavour and a high free acidity, the latter reducing the smoking temperature of the fat (page 44) and thus causing trouble in frying. The resistance to oxidative rancidity of fats of both types is low, and excessive softness in lard is a disadvantage when it is used as shortening. A combined process of deodorization, neutralization and slight hydrogenation converts these products into materials of better grade and improved keeping quality. It is said to be becoming common practice in the chocolate industry to reduce the iodine-value of cacao-butter by one or two points in order to reduce the tendency to "blooming" in the finished chocolate[10].

TABLE 48.—*Selective hydrogenation of linoleic to oleic glycerides in cottonseed-oil.*

(Hilditch[132])

Iodine-value of oil	Component acids (%)			
	Oleic	Linoleic	Total unsaturated	Total saturated
109·1	23·8	51·5	75·3	24·7
100·8	34·5	40·5	75·0	25·0
96·1	40·5	34·5	75·0	25·0
77·4	60·0	15·0	75·0	25·0
72·1	62·0	11·0	73·0	27·0
65·2	68·5	3·5	72·0	28·0
57·5	67·0	0·0	67·0	33·0

In Table 48 are given figures for cottonseed-oil which show very clearly the selective nature of the process of hydrogenation. These, and the data previously quoted for lard, refer to the "batch" and to the "overflow" (continuous) processes, in which the vessel containing the catalyst is maintained practically full of oil in rapid motion. In the "drip" modification of the continuous process, in which the oil is allowed to percolate in relatively thin films over the catalyst in an atmosphere of hydrogen, hydrogenation is much less selective, and the proportion of stearic glycerides present increases steadily from the commencement of the run. The formation of *iso*oleic glycerides is, however, considerably reduced. Recent work indicates that selective hydrogenation is not universal, even with the "batch" process. Thus, the complex, highly unsaturated glycerides of whale-oil are said to be converted on hydrogenation first to the diethenoid stage, and thence directly to saturated acids. Further, in the case of rape-oil, which contains 50 per cent. of

erucic ($C_{22}H_{42}O_2$), 30 per cent. of linoleic and 15 per cent. of oleic acid, hydrogenation appears to be much less selective than in oils in which oleic and linoleic are the chief unsaturated acids[132].

The Continuous Process

In the original or " batch " process of hydrogenation the oil is agitated with the required amount of gas under pressure, usually at a temperature of about 180° C., in the presence of a relatively small quantity of a highly active, finely divided catalyst. The latter is produced in this case by the deposition of nickel carbonate on a supporting material (kieselguhr), followed by careful reduction in a stream of hydrogen at 300°–350° C. The process, including the heating and cooling of the oil, filtration, etc., requires several hours for completion.

More recently a continuous process has been introduced. The catalyst in this case consists of nickel gauze or turnings, the surface of which is activated by anodic oxidation and subsequent reduction in a stream of hydrogen. The oil and hydrogen pass over the catalyst contained in a series of cylinders, the degree of hydrogenation being controlled by adjustment of the rate of flow. When only a slight degree of hardening is required, the output can be high. Filtration is not necessary, and the catalyst, which lasts well, can readily be reactivated.

The quantity of gas required for hydrogenation is small; 0·68 per cent. by weight, for example, will convert liquid triolein into tristearin, a hard solid which melts at approximately 71° C. Alternatively, 34 cu. ft. of hydrogen are required to reduce the iodine-value of one ton of oil by one unit.

SECTION 8.—THE YELLOWING OF FATS AND OILS

Closely connected with the process of oxidation is the phenomenon of " yellowing " sometimes observed in unsaturated oils and fats and in soaps. This consists in the development on storage of a colour varying in intensity from a very pale yellow to a dark orange or reddish-brown. Several cases are of importance in the food industry.

Pork and Bacon

When a side of bacon is allowed to hang at room temperature in an atmosphere sufficiently dry to prevent spoilage by moulds and bacteria, the exposed surface of the fat becomes discoloured, first cream, then yellow (usually in patches of varying intensity), finally reaching a deep orange yellow in very old samples. The production of this colour is apparently linked in some way with oxidation, since fat which has turned yellow always shows a high content of peroxide oxygen[181, 183]. Oxidised pig's fat, on the other hand, is not invariably yellow. Exposed fat on sides of bacon which had been stored at −10° C. for five months was only pale cream in colour, in spite of a peroxide content of 60 ml. per gram., but rapidly became yellow when brought up to room temperature. Yellowing,

however, does occur at low temperatures after storage for sufficiently long periods. At 15° C. the fat was already distinctly yellow at peroxide values as low as 15 ml. per gram.

Pig's fat separated from the tissue does not become yellow on oxidation*, but the addition of a trace of alkali—sodium hydroxide, trimethylamine or ammonia—to oxidised (colourless) lard rapidly produces a yellow colour[185].

Rabbit's Fat

The yellowing which occurs in the fat of wild rabbits held in cold storage for long periods has been investigated by Vickery[304,305], who finds that in this case also the colour is due to oxidation. Rabbit's fat (in the tissue) stored under nitrogen for 12 months at −5° to −19° C. failed to develop any colour, while control samples stored in air became deep yellow. Oxidative changes in rabbit's fat (iodine-value, 117–179), as in the fish-oils mentioned below, are, of course, much more marked than in the fat of bacon (iodine-value, usually 55–70) under similar conditions. On the average, a distinct yellow colour was found to appear within 60 days, and pronounced yellowing after 120–150 days at −10·5° C. At −18° C. the corresponding periods were 120–150 days and 270 days respectively.

Vickery concluded that the colour arises from the oxidation of the linoleic glycerides, and suggested that it might be due to the formation of a yellow unsaturated ketone. Since the addition of a small quantity of minced tissue or tissue-extract to pure rabbit's fat or cottonseed-oil produced yellowing, he further concluded that a tissue-oxidase takes part in the process. The data presented are not in themselves conclusive, since subsequent work (page 190) indicates that protein or nitrogenous bases in the tissue or extract would be sufficient to produce a yellow colour with oxidised fat. Nevertheless, results recently obtained with herring-oil (page 222) and with pig's fat (page 216) make it very probable that an enzyme *does* accelerate oxidation of the fat of the rabbit, and hence give rise to the conditions necessary for the development of the yellow colour. It has not been proved that an enzyme is necessary for the production of the yellow colour from oxidised fat.

The "Rusting" of Fish

Related to the cases mentioned above is the phenomenon of "rusting" observed at ordinary temperatures in dried, salted fish, or after cold storage in fish such as herring, salmon or mackerel in which the muscle contains a large proportion of highly unsaturated oil. The oil on and immediately beneath the skin, and particularly round the bases of the fins and on the exposed muscular surfaces of gutted fish becomes gummy and reddish-brown in colour, and the flesh has an unpleasant rancid flavour when cooked. Dutch workers[8], by experiments on storage in atmospheres of nitrogen

* Except, perhaps, after storage for very long periods.

have established the fact that rusting also is essentially an oxidative phenomenon.

The presence of traces of ammonia in the air of refrigerated chambers has been found to increase the rate of rusting of frozen fish. Oxidised oils recovered from rusted fish by extraction with a solvent always contain appreciable quantities of combined nitrogen[32]. It has therefore been suggested that the primary cause of rusting may be the action of a volatile base produced by bacterial enzymes on the (presumably oxidised) oil[19]. Ammonia and trimethylamine are known to be formed by the action of bacteria on fish's muscle[9], and trimethylamine oxide, which also rapidly darkens oxidised fat[68], is a normal constituent of the muscle[118].

Recent investigations by Davies and Gill[68] have shown that the colour of cod-liver oils increases progressively with the amount of combined nitrogen present, figures obtained ranging from 0·014 per cent. for pale medicinal oils to 0·111 per cent. for a brown industrial oil. The very dark brown ether-extract from fish-manure contained 0·876 per cent. of nitrogen. Experiments by these workers also showed that the atmospheric oxidation of unsaturated fats in the presence of nitrogenous substances such as casein, lecithin, or trimethylamine oxide, leads to the combination of nitrogen with the fat and progressive darkening.

The Yellowing of Films of Drying Oils

The process of yellowing, as observed in the fats of food has a number of points in common with a similar discoloration which often develops in white paint and enamel, particularly on interior surfaces, behind pictures, etc., where it is not exposed to bright light. This fault, which is one of some importance in the industry, has been investigated by several workers, using films of linseed- or tung-oil[309, 262], or of pure triglycerides.[262, 79, 215] Yellowing, which is much more pronounced with some oils (linseed) than with others (poppyseed), is retarded by exposure to light. A film which has become yellow in the dark may even bleach again on exposure to light, but with extreme age yellowing occurs even in the light. Moisture and a temperature above zero appear to be necessary for the production of the colour, but the presence of oxygen is not[309], which indicates that the change is due to the rearrangement of compounds already present (peroxides), rather than to further oxidation.

Morrell and Marks[215], from a study of the products of the autoxidation of β-elaeostearin, have come to the conclusion that the production of colour in this case, at least, is due to the action of traces of alkali on one of the peroxide groups (that nearest to the glyceryl residue) which in its enolic form is said to be acidic and to form coloured salts. Disruption of the further peroxide which also occurs, particularly in the presence of alkali, is considered to be a contributory factor.

Schieber[262], on the other hand, believes that the colour produced in films of drying oils is due to polyketones, formed by intramolecular rearrangement of the original peroxides (page 89). Two or more adjacent CO groups in a molecule are known usually to result in a yellow or orange compound. This theory has been adopted by Elm and Standen[81] who conclude from measurements of the absorption-spectrum that compounds of the type suggested by Morrell and Marks would not be coloured. A further possibility suggested by Elm[80] is that the yellow colour might be due to the resinifying action of traces of alkali on aldehydic products of rancidity.

Yellowing in the fat of foodstuffs therefore seems to occur as a result of the action of traces of basic substances on fat peroxides, or by the combination of tertiary nitrogenous compounds with the oxidised fat in some manner not at present understood. Trimethylamine oxide is already present in some foods, and trimethylamine, ammonia, etc., can readily be produced from phosphatide or protein by the action of bacterial or tissue enzymes. Trimethylamine may also arise directly from the autoxidation of lecithin in the presence of traces of iron or copper.

Alkali or nitrogen does not, however, appear to be essential in every case for the production of a yellow colour in an oil or fat. Thus, the highly unsaturated liquid esters obtained during the analysis of a fish-oil by the ester-fractionation method, often distil over yellow in colour or develop a deep yellow tint on keeping. A similar colour has been obtained by heating methyl linoleate in air at 100° C. for some hours, and even a sample of lard, stored in a dark cupboard for several years, has been found to possess a distinctly yellow colour. Heat alone without further oxidation may sometimes cause yellowing in a very old and rancid oil[185]. In all of these cases no alkali was present, other than possibly a trace derived from the glass container.

Yellowing by the Agency of Micro-organisms

Of an entirely different nature to the change discussed above is the discoloration produced by various types of micro-organisms growing on media containing fat. Thus, *Penicillium* can give rise to bright yellow patches on the adipose tissue of beef stored at 0°C., without the mould itself being visible to the unaided eye, and yellow colours have been observed as a result of the growth of bacteria on lard in the presence of a nutrient. One such yellow pigment produced by a specific type of micro-organism is said to function as an oxidation-reduction indicator, and turn bluish-purple in the presence of fat peroxide, thus producing the characteristic discoloration sometimes observed on hams, etc[161]. Pigments produced by micro-organisms are, however, usually described as structurally related to the carotinoids rather than to the fats.

SECTION 9.—"FISHINESS" IN FAT-CONTAINING FOODS

Fats and fat-containing foods such as lard, butter, cream, dried milk, liver, bacon, and dried egg-yolk occasionally acquire an unpleasant flavour suggestive of stale fish-products.

In milk, a taint of this kind is sometimes found when the cows have been fed beet-tops and other by-products of the sugar-beet industry which contain appreciable quantities of betaine (trimethyl glycine). Even young lucerne and red clover, which contain considerable amounts of choline, tend to produce a fishy taint when fed in large quantities[65]. The cow metabolises the tertiary nitrogenous bases of these foodstuffs to trimethylamine oxide, traces of which may pass into the milk[235]. Davies believes that the fishy flavour of such milk is actually due to compounds produced by the interaction of trimethylamine oxide with the double bonds of the unsaturated fat[66]. Certain factors inhibitory to the oxidation of fat which vary with the diet (page 204) and with the individual cow, cause the taint to appear with widely different intensities in different samples of milk[65].

Fishy flavours have frequently been observed in pork, bacon and hams after feeding to the animals whale-oil, low-grade fish-meal or cod-liver oil. The taint in the fat of the fresh pork, if noticeable at all, is slight,* but becomes marked when the pork is stored for long periods, or on keeping after conversion to bacon. In this case the fishy flavour is usually superimposed on the ordinary rancid flavour of the oxidised fat. The source of the defect is probably the laying down by the pig in its body-fat of some of the highly unsaturated fatty acids absorbed from the marine animal oils of the food (page 26). The presence of these acids seems to cause a predisposition to the development of "fishiness," though the manner in which the flavour is produced is still not fully understood. Some progress, however, has been made towards its elucidation.

Mechanism of the Production of Fishy Flavour

The reason for the characteristic odour and flavour of the fish-oils themselves is by no means clearly established. Tsujimoto nearly 30 years ago stated that it is due to the presence of glycerides of clupanodonic acid ($C_{22}H_{34}O_2$) and presumably of other highly unsaturated acids which accompany it. Certainly very slight hydrogenation destroys the odour, which, however, tends to reappear on keeping in contact with air. Refined fish-oils, e.g., cod-liver oil, can also be obtained practically odourless and flavourless, but again fishiness reappears to some extent with the development of rancidity. It would seem therefore that the flavour may be due rather to products of oxidation than to the acids themselves.

Fishiness in butter, dried milk or cream has usually been attributed to the production of trimethylamine from lecithin, which is

* A slight but definite "oily-fishy" flavour has been observed in extracted fat from freshly killed pigs which had received a diet containing cod-liver oil [197].

present in milk and butter to the extent of 0·03–0·12 per cent. and 0·01–0·17 per cent. respectively. The formation of trimethylamine from lecithin involves the rupture of a C-N linkage, and is therefore not likely to be due to a simple hydrolytic process, as suggested by the earlier observers[58, 274]. Choline, $CH_2OH.\ CH_2.\ NMe_3.\ OH$, the nitrogenous constituent of lecithin, can, however, be broken down by suitable oxidising agents with the production of trimethylamine, and this substance has been obtained in appreciable yield by the action of hydrogen peroxide in the presence of a ferrous salt on both choline and lecithin[64].

It seems possible therefore that one of the mechanisms responsible for the production of fishiness may be the action of peroxides present in the oxidising fat on the choline residue of the lecithin molecule. The presence of copper or iron would favour the development of the taint, both by accelerating the formation of the fat peroxides, and by assisting their action on the lecithin. Many cases of fishiness in dairy-products have been traced to metallic contamination of the milk (page 205). It must be admitted, however, that certain other fatty products which contain more lecithin than butter show much less tendency towards development of the taint.

Davies and Gill[68] have recently showed that when fish's muscle is allowed to autolyse, or the fat of dried fish to oxidise *in situ*, the colour of the extracted oil and the intensity of its fishy flavour increase in a manner roughly proportional to the amount of nitrogen bound by the oil. Thus, very pale, almost tasteless medicinal oils contained only 0·014 per cent. of nitrogen, which increased with colour and flavour to 0·111 per cent. for a very brown and highly flavoured industrial oil, and to 0·876 per cent. for the very dark brown and intensely fishy extract of fish-manure. Roughly 50 per cent of this nitrogen could be extracted by dilute acid, and gave reactions characteristic of trimethylamine.

Fishiness was reproduced experimentally in butter-fat and in linseed- and olive-oils by permitting the fat to autoxidise for some weeks, preferably with a salt of copper or iron as a catalyst, in the presence of a source of nitrogen such as betaine, lecithin or casein. In all cases appreciable amounts of nitrogen entered into chemical combination with the oil, and an unmistakably fishy flavour and a yellow or brown colour were produced. The reaction in the case of betaine and of lecithin was apparently a Fenton oxidation by fat peroxide in the presence of the metal, with rupture of the C-N linkage to produce trimethylamine. 90 per cent. of the volatile base-nitrogen recoverable from the oil was in forms other than ammonia. The reaction between oxidised oil and casein also led to the production of appreciable quantities of volatile bases, possibly by interaction between aldehydes produced in the oxidation and amino nitrogen, with the formation of aliphatic amines.

Trimethylamine oxide, NMe_3O, which can be prepared by the action of hydrogen peroxide on trimethylamine, is known to be a

normal constituent of fish's muscle[118, 9], and may possibly occur also in the tissues of other animals and in milk[63]. This substance by heating with various oils and fats for several hours at 105° C., could be reduced with the liberation of a certain amount of free trimethylamine, while the oils acquired a fishy odour, a brown colour, and a content of combined nitrogen of from 0·04 to 0·10 per cent.

These experiments by Davies certainly throw new light on the development both of "fishiness" and of the yellow discolourations in edible fats, but further work is still necessary to determine the manner of combination of the bound nitrogen, and to ascertain whether or not enzymes play any part in the development of these defects in the tissue.

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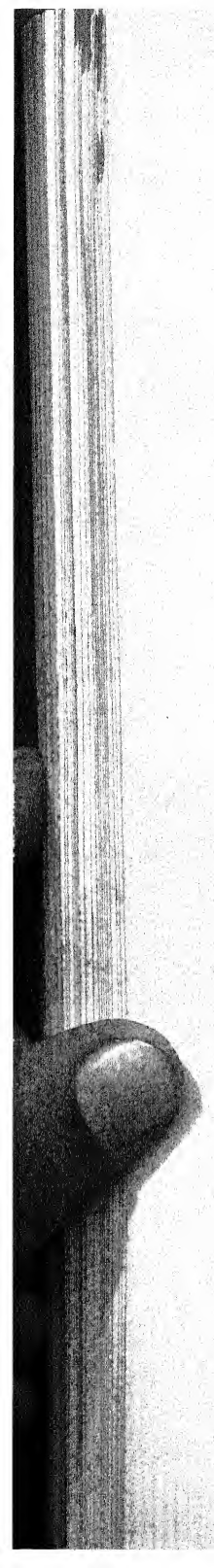

PART VI.—RANCIDITY IN DAIRY-PRODUCTS AND IN THE FAT OF MEAT

In the foregoing pages the deterioration of fat has been considered from the viewpoint of the type of chemical change involved, rather than from that of the individual foods affected. The present section is intended to illustrate these data by brief reference to the types of deterioration encountered in a few of the more important fat-containing foods.

Dairy-products.

Milk

Milk is subject to many taints and off flavours. Some, present in the freshly drawn milk, may be due to the presence of garlic, onion, mustard, chamomile or thyme in the pasture, or to foods such as cabbage, beet or turnips, particularly when fed shortly before milking. Taints from these sources are usually stronger in the cream than in the milk, and butter made from such cream is nearly always tainted. In addition, the aroma and flavour of drawn milk is easily impaired by the absorption of odours from stable-manure, paraffin, disinfectants, etc. Aeration of the milk during processing reduces strong flavours due to the feed, and eliminates slight taints from both causes.

Natural enzymes present in milk give rise to a variety of defects. A bitter flavour and burning sensation at the back of the mouth have been found to be due to an abnormal amount of lipase in the milk[62]. Usually only a few cows in a herd are affected, and the defect is not continuous, but appears and disappears from time to time[65]. Milk from old cows and from cows at an advanced stage in lactation is particularly liable to be affected. An oily flavour, changing subsequently to a sharp, rancid or even soapy taste, has also been attributed to the activity of fat-splitting enzymes in the milk[19]. Homogenisation, by increasing the area of the globules of fat exposed to enzyme action, accelerates the development of rancidity from this cause[29]. The lipase normally present in raw milk (page 45) may survive the preparation of products such as cream, butter and condensed and dried milks, and cause deterioration in these articles during storage. Addition of as little as 0·75 per cent. of unheated milk to a batch of sweetened condensed milk has been found to cause rancidity, though the amount of lipase present in this case corresponded only to 0·3 per cent. of that originally present in the milk. Pasteurization destroys the enzyme, and is invariably resorted to in the preparation of dairy-products intended for storage.

Similar defects develop in milk or cream, owing to the activity of lipolytic organisms. Many of these also attack the casein, and cause a deterioration in flavour which is partially due to products of the degradation of protein. An interesting type of microbial

spoilage is the "coconut" or "carbolic" taint encountered in commercial sterilized milk[40]. Affected samples taste acid and bitter, have a smell reminiscent of coconut-milk, and give positive reactions for methyl ketones and for phenols. Apparently, ketone rancidity of the fat and breakdown of protein (tyrosine) are both involved, the organism responsible being a facultatively anaerobic bacillus whose spores resist the 30 minutes at 99°–100°C. employed for sterilisation. Numerous other types of "off" flavour and odour resulting from the proliferation of micro-organisms in milk are associated mainly with constituents other than the fat, and will not be discussed here.

Oxidation of the fat or lecithin[82] of milk by free oxygen produces flavours which have been variously described as "oily," "cardboard," "cappy," "emery," etc. The immediate cause is usually some factor which accelerates the normally slow process of oxidation. Recent work[42] suggests that an enzyme present in raw milk can produce an oily, oxidative taint. This defect occurs mainly in the winter months[33], though there may be some delay in the disappearance of the flavour in the spring and its advent in the autumn.[3] A high titratable acidity in the milk favours development of the taint[3]. Fresh, green pasture[14], hay[42, 2], carrots[2], or soya beans[64] in the diet reduce the tendency to development of an oxidised flavour, possibly owing to the transference of natural antioxidant from the food to the milk. The feeding of orange- or lemon-juice or of vitamin C (0·5g. of ascorbic acid daily), to the cow, or the addition of ascorbic acid (100mg. per litre) to the milk, greatly reduces susceptibility[14, 73]. Incubation of the milk or addition of cultures of reducing bacteria has the same effect[42, 84]. Pasteurization at 63°C. for 30 minutes fails to give protection, but the enzyme is destroyed by heating at 85°C. for 5 minutes[42], or at 77°C. for 10 minutes, exposure to somewhat lower temperatures considerably reducing its activity[73].

Exposure to direct sunlight for as little as 10 minutes causes a distinct flavour in milk, and exposure for 45 minutes a strong tallowiness[37]. Eight hours' exposure to diffused daylight at 0°C. has been found to result in a "cardboard" taste and a "linseed-oil" flavour[32]. Taints of this kind develop more rapidly in pasteurized than in raw milk, and are not due to the presence of enzymes or bacteria.

The harmful effect of light on milk-fat raises the question of the stability of the fat during the reinforcement of the vitamin in milk by irradiation. Ultra-violet light of wavelengths between 2,300 and 3,130 Å converts certain of the sterols present in milk into vitamin D. Thus, exposure of milk as it flows in a thin film over the cooler to a source of ultra-violet light increases the antirachitic value by from 5 to 50 times[18]. In more recent work, milk containing 1·2 per cent. of fat has been exposed to carbon arcs for periods up to 16 seconds, resulting in a 12-fold increase in vitamin D activity. A slight destruction of vitamin C apparently occurs under these conditions, but exposures up to 48 seconds have no effect on the vitamin

A content[39]. Irradiation for more prolonged periods fails to increase the vitamin D[78], but reduces vitamin A, destroys vitamin C, and causes a definite taint. Anderson and Triebold[1], however, state that the irradiation of milk for periods as long as 120 seconds only reduces the induction-period of oxidation of the fat by about 15 per cent., and conclude that exposure for the normal period (15 seconds = 2 million ergs per ml.) should have no appreciable effect. Other workers[89] definitely associate the unpleasant flavours produced by excessive irradiation with changes in the protein (Table 49). On the other hand, the "burnt" ("activated") and "tallowy" taints caused in milk-products by exposure to sunlight appear to be distinct flavours attributable to changes in the protein and the fat respectively, the former tending to predominate in material of low and the latter in material of high fat-content. Whole milk exposed to direct sunlight may exhibit both flavours simultaneously[28]. The use of an atmosphere of carbon dioxide during irradiation is said to prevent the development of taint in either protein or fat[71].

The commonest cause of oxidative taints in milk-fat is contamination with traces of copper or, less frequently, with iron. Machinery for dairies, particularly pasteurizers and coolers, is usually made of copper, a metal which is easily worked and possesses excellent thermal conductivity. Surfaces which come into contact with the milk are protected from corrosion by a heavy coating of tin which, as already pointed out, is one of the least harmful of metals from the point of view of oxidation of fat. If, as a result of continued use, portions of the protective coating become abraded, exposing the underlying metal to the solvent action of the milk, harmful amounts of copper can easily be taken up. The taint produced consists of an odour suggestive of castor-oil and an oily, nauseous taste, which usually develops after the milk has been standing for from 12 to 36 hours. It occurs most readily in samples of low bacterial count, and at low temperatures. This generalisation, which holds also for oxidative taints produced by light, depends upon the fact that a rapidly proliferating micro-flora maintains the oxidation-potential in the milk at so low a value that oxidation of the fat cannot occur.

The quantities of metal necessary to produce taint are small. Milk containing 1·5 parts of copper per million develops an oxidised flavour in about 24 hours at 0–5°C.[22], and yields a dried product containing 10–15 parts of copper per million which becomes tallowy abnormally rapidly on storage[76]. Stainless steel is now being used to a considerable extent in place of tinned copper for the manufacture of equipment for dairies. Recent work on the corrosion of metals and alloys by milk has been reviewed by Davies[24].

Dried Milk

Freshly prepared milk-powder contains comparatively few bacteria and very little moisture, and, unless allowed to take up large amounts of water from the atmosphere, the few organisms present decrease in number on storage and no spoilage from this

TABLE 49.—*Flavours produced in individual constituents of milk by exposure to the radiation of a quartz-mercury lamp (a) through 2 mm. of quartz (cut off 2,000 Å) and (b) through 1·5 mm. of window-glass (cut off 3,100 Å).*
(Weckel and Jackson[87])

Constituent	Quartz (exposure in mins.)	Flavour	Glass (exposure in mins.)	Flavour
Whole milk	10·5	Activated	150	Slightly activated
Butter-fat	50	Oily	200	Tallowy
Casein	8	Activated	400	Normal
Albumin	8	Activated	450	Normal
Casein-free whey	12	Activated	400	Normal
Casein- and albumin-free whey	45	Very slightly activated	420	Normal
Solution of lactose	120	Normal	—	—
Skim milk	11	Activated	420	Normal

cause occurs. Dried whole milk is, however, very susceptible to spoilage through oxidation of the fat, whereby the pleasant, fresh aroma and flavour are quickly lost, and the powder becomes stale and tallowy. The amount of water present appears to play some part in determining the course of oxidation (page 181), samples of water-content higher than about 3·5 per cent. showing first a fishy and then a tallowy flavour, while drier samples became tallowy without acquiring the intermediate fishy flavour[26]. The influence of copper and of light on oxidative spoilage, and the effect of lipase on the development of hydrolytic rancidity, have already been discussed. Milk-powder is frequently packed in containers sealed under vacuum or inert gas, whereby a considerable improvement in storage-life is obtained.

Butter

The flavour of butter made from fresh cream, though pleasant, is very mild, and is commonly enhanced by " ripening " the pasteurized cream with a culture of suitable bacteria prior to churning. The characteristic aroma and flavour of butter produced in this way is due mainly to diacetyl, $CH_3.CO.CO.CH_3$, which, with its precursor acetyl methyl carbinol, $CH_3.CHOH.CO.CH_3$, is formed by the bacteria during the ripening. The period of ripening is frequently much curtailed, and may even be eliminated altogether, the aromatic constituents then being added preformed in the butter culture. The amount of diacetyl present in butters of mild, medium and full flavour is of the order of 0·05–0·3, 0·4–0·8, and 0·9–2·0 parts per million respectively.* Increasing the diacetyl beyond 2·5 parts per million by the addition of the synthetic substance produces a butter of repulsive flavour[25].

Though ripening the cream increases the desirable flavour of the butter, keeping quality is also impaired, and butters of full flavour tend to deteriorate abnormally rapidly on storage. Part of this effect is undoubtedly due to the increased acidity developed during ripening, and the acidity of ripened cream is therefore frequently reduced by neutralisation with sodium bicarbonate or with lime before churning. Butter intended for prolonged storage is usually churned at an acidity of 0·10–0·13 per cent. (as lactic acid in the cream), higher values (*e.g.*, 0·25 per cent.) yielding butter which becomes fishy on storage[26]. Neutralisation must not be carried too far, since an alkaline *p*H favours decomposition of the protein and reduces keeping quality[74, 11].

Some evidence has been obtained that diacetyl itself has pro-oxidant properties. Thus, samples of butter-fat enriched with amounts of diacetyl greatly exceeding those normally present in butter become bleached and tallowy more rapidly than untreated controls[44]. Margarine whose odour and flavour has been improved by the addition of diacetyl[79] and butters of high diacetyl-content[35] are both said to lose their aroma and become tallowy abnormally

* In a more recent report[4] these values are reduced to 0·05–0·2, 0·3–0·6 and 0·7–1·2.

rapidly on storage. On the other hand, the addition of four parts per million of diacetyl to butter has been found not to affect the keeping quality, which was related to acidity but *not* to diacetyl-content[10].

The pleasing aroma, flavour and texture of fresh butter deteriorates from a variety of causes during storage. Spoilage through the absorption of odours, enzymic changes, and the formation of ketone by micro-organisms have already been discussed in some detail. "Rancidity," by which in dairy-produce is usually meant the production of free butyric and other volatile acids, is caused by *Pseudomonas fluorescens, Oidium lactis* and other powerfully lipolytic organisms[27].

Faults such as "cheesiness" and "surface taint" are due essentially to the activity of proteolytic organisms, though considerable hydrolysis of the fat may also occur. *Achromobacter putrefaciens* and *Pseudomonas fluorescens* have been found to be commonly associated with the production of "surface taint"[27, 72], a form of spoilage in which a putrid odour and flavour develop in the superficial layers of the butter. Minster[58] has described methods for classifying unsalted butters according to potential keeping quality, by estimation of catalase and reductase, together with a simple bacteriological examination and incubation test. Microbial spoilage in general is much less common in salted than in unsalted butter.

Mould frequently causes discolourations on butter and on the parchment liners of butter-boxes, particularly in places where the contents have shrunk away from the sides of the box through drying. The colours most usually developed are black, brown, green, yellow, orange and red, due mainly to species of *Stemphylium, Alternaria, Cladosporium, Mucor, Aspergillus, Penicillium, Oidium* and *Fusarium*. Moulds are usually said not to grow under completely anaerobic conditions, but some of them require extremely little oxygen. Thus growth on butter is usually confined to within a few millimetres, or at most one or two centimetres, of the surface; occasionally it follows cracks and fissures further into the interior. Superficial growths of this kind can be removed fairly readily with the outermost layers of the butter, but the flavour of the underlying material has frequently been impaired by the diffusion of highly flavoured products of decomposition from the surface. Unsalted or slightly salted butter is sometimes completely spoilted by *Oidium lactis*, which grows down into the interior of the mass, often showing little or no visible growth on the surface. Black spots due to *Cladosporium herbarum* are occasionally found in the interior of unsalted, and more rarely of salted, butter.

Spores of moulds are invariably present on the wood of butter-boxes, and a double layer of ordinary parchment is ineffective in preventing their access to the butter[67]. Parchment backed by aluminium foil prevents moulds from penetrating from infected boxes to the surface of the butter. This type of wrapper is also very

effective in decreasing desiccation, which deepens the colour of the surface and produces a streaky appearance when the butter is made into smaller prints or used for blending, and in preventing tainting by the absorption of odours (page 39). The treatment of wood or fibreboard boxes with salicylanilide has given good results in controlling spoilage by mould.

The taints produced by moulds vary from the sharp, butyric acid odour of the hydrolysed fat, and the " Roquefort cheese " odour of methyl ketones, to mustiness and the cheesy and putrid odours of decomposed protein.

Notwithstanding the relatively high water-content of butter (11–16 per cent.), the growth of mould on its surface is influenced considerably by the humidity of the atmosphere. Thus, butter inoculated with spores and stored at room temperature became mouldy with decreasing rapidity at 100, 90 and 80 per cent. relative humidity, and at 70 per cent. no growth occurred[81]. This is probably due to the fact that in butter, fat is the continuous phase and moisture is transferred only slowly from the interior to the surface to replace that lost by evaporation. Growth continues even at low humidities when the mycelium has already penetrated into the butter[57].

Microbial spoilage of butter is limited to storage at atmospheric or at chilling temperatures. Butter intended for long storage is held at $-10°$ C. or below, which completely inhibits microbial growth. Nevertheless, hard-frozen butter definitely deteriorates in palatability if held for sufficiently long periods, the most marked taints developed being metallic or fishy flavours and, ultimately, tallowiness. Chilled storage is not successful, the flavour rapidly becoming stale and tallowy[8].

Fishiness, which has already been discussed (page 192), results either from the production of trimethylamine by the hydrolytic and oxidative decomposition of lecithin, or from the interaction of nitrogenous substances such as protein with oxidising fat. The taint occurs most frequently in salted butter of full flavour made from acid cream, and particularly in butter contaminated with copper or iron. Overworking the butter is said to increase the tendency towards fishiness, probably owing to the incorporation of excessive amounts of air, and there is some evidence that low contents of air favourably influence keeping quality[5]. It has been stated that fishiness can be produced in evaporated milk, cream and butter by the agency of a specific bacterium[36, 20], and that this organism forms trimethylamine from lecithin[77]. It is certain, however, that the taint frequently develops in butter at temperatures much too low for microbial action.* Tallowiness, which in very

* It is possible that an enzymic oxidising system hastens the change. If this be so, pre-heating the milk to a temperature considerably above that normally employed for pasteurization should reduce the tendency of the butter to develop fishiness. This has recently been claimed to be true[68] Further work is necessary to assess the importance of oxidising enzymes as possible accelerators of the oxidative deterioration of butter.

advanced cases is accompanied by bleaching of the superficial layers, also develops at cold-storage temperatures, particularly in butters contaminated by metals. In such cases a taint which appears first as "metallic," "oily" or "fishy," frequently becomes "tallowy" on further storage. Metallic contamination is usually attributable to machinery used in manufacture. Parchment papers used for wrapping contain traces of copper and iron, but apparently not sufficient to accelerate appreciably the oxidation of butter in contact with them[23]. Tallowiness is also produced very readily by exposure to daylight, and particularly to direct sunlight. In the latter case a few minutes is sufficient to spoil the flavour.

Defects less clearly defined than these are usually grouped as "storage" flavours, and it is by no means certain to what processes they are due. Freshly made butter contains up to 10 per cent.[67] (normally *ca.* 5 per cent.) by volume of entrapped air, and during storage the oxygen of this air slowly disappears, indicating that oxidative changes of some kind are taking place. These include the incipient oxidation of lecithin or fat already referred to, but probably more important are oxidative processes involving non-fatty constituents of the butter. Changes of this kind are said to be greatest in butter of high curd-content, and to be proportional to the acidity of the cream from which the butter is made. Thus, it has been found that the off flavour developed during storage for six months at −17·8° C. in butter made from (*a*) sweet cream of acidity 0·11 per cent., (*b*) cream churned immediately after the addition of starter to an acidity of 0·25 per cent. and (*c*) cream containing 0·71 per cent. of lactic acid was negligible, slight, and very pronounced respectively[30]. The changes in oxygen-content of the enclosed air in the three cases were 25·2 to 25·1, 10·9 to 9·0, and 21·6 to 4·2 per cent. (in three months). Recently, attempts have been made to control these changes by working and packing the butter (or margarine) in an inert atmosphere, and it has been claimed[38], in opposition to earlier results[41, 66], that the keeping properties at low (but not at atmospheric) temperatures are improved about three times by this process.

Stale or rancid butter is sometimes "renovated" as follows. The butter is melted in tanks at 40°–45° C. and the separated curd and brine run off. The clear fat is then aerated to remove objectionable odours, churned with whole or skimmed milk, and chilled by running into ice-cold water. Finally the product is drained, allowed to "ripen" or develop a flavour, worked to free it from excess of water, salted and packed.

Meat.

The commercial storage of meat and fish provides examples of most types of the spoilage of fat, the particular mechanism responsible varying with the nature of the product and the conditions of storage. The amount of scientific data available is still small.

Frozen Beef and Lamb

Lean meat (muscle) begins to freeze at about —1° C., and as the temperature is reduced, progressively more of the 75 per cent. of water present becomes converted to ice. In fresh meat this process is still not quite complete even at —20° C., and in bacon the percentage of water frozen out is less, owing to the presence of the salt. The separation of ice increases the hardness of the frozen carcases, and leads to the formation of " drip " on thawing. Meat held at —5° C. and below is usually referred to as " frozen," while at —2° C. and above it is classed as " chilled."

Meats commonly stored for long periods in the frozen state include mutton and lamb, beef, pork, chickens, turkeys, geese, etc., and rabbits, the temperature employed being usually in the neighbourhood of —8° to —10° C. Under these conditions microbial spoilage is completely inhibited, but tainting will, of course, occur after thawing if the meat is exposed to high temperatures and humidities.

The fats of beef and mutton are comparatively resistant to oxidation. Changes in the external fat of carcases of lamb have

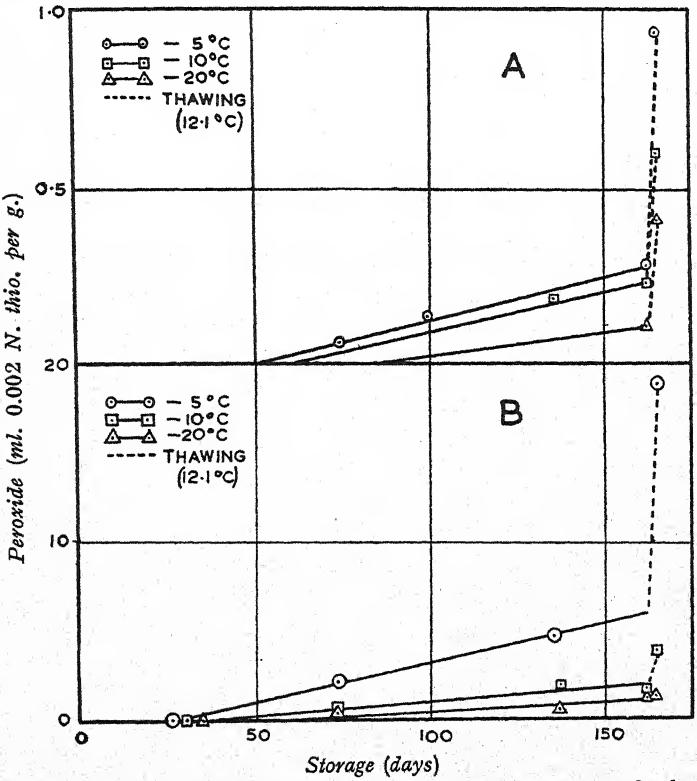

FIG. 34.—*Oxidation of the superficial fat (0·5 mm.) of frozen lamb during storage. (A) stored without sweating; (B) sweated.* (Lea[45])

been found to be small, even after seven months at −5° C.[45], and meat placed at −10° C. immediately after dressing was still in good condition as regards the palatability of the fat 18 months later[48]. In both of these cases the meat remained palatable for at least three days at atmospheric temperature (12° C.) subsequent to storage. On the other hand, partial thawing of the surface, as, for example, during transference from rail to store, from store to ship and back again—particularly if allowed to occur in strong daylight—may increase the tendency of the meat to become tallowy during marketing. The data given in Fig. 34 refer to carcases of lamb stored at −5°, −10° and −20° C. for five months, and subsequently held for three days in subdued daylight* at 12·1° C. "Sweating" was for seven periods of six hours each at 10·4° C. in subdued daylight, the periods being distributed uniformly throughout storage. Changes in free acidity in this experiment were very slight (Table 18), and only on the carcases stored at −5° C. were any visible colonies of micro-organisms (moulds and yeasts) observed; these were not on the fat.

Pork

The fat of pork (iodine-value 55–70) is more unsaturated and less stable than that of beef or mutton (iodine-value 35–47). Pork can, nevertheless, be stored successfully in the frozen condition, and considerable quantities of frozen pork are imported into this country from Australia, New Zealand, the U.S.A. and the Argentine. The smaller carcases are sold as pork and the larger used for the manufacture of bacon. Bacon of good quality can still be made from frozen pork after storage for several months.

Chickens

The fat of the domestic fowl, which represents a further stage of unsaturation (iodine-value 70–90), can still be stored for a few months in the frozen state without very marked deterioration. Birds hard frozen for long periods, however, develop the unpleasant acrid odour and flavour which accompanies the autoxidation of fat, and positive reactions for aldehydes have been obtained by means of Schiff's test on birds frozen for from two to seven years[63].

Rabbits

With rabbits, which have fat of iodine-value of the order of 110–180, oxidative spoilage becomes of immediate practical importance. In the gutted, frozen carcase the fat surrounding the kidneys and covering part of the wall of the abdominal cavity is freely exposed to the air and oxidises rapidly, developing ultimately an acrid, rancid odour which penetrates and spoils the neighbouring muscular tissues. Spoilage to this degree is usually present when the period of storage exceeds two months at −5° C., or four months at −10° C.[86] A strong Kreis test has been observed in the fat of rabbits (*a*) stored at 10°–20° C. for 44 hours prior to freezing and (*b*) frozen immediately, after respectively 1 and 2 months at −5° C.,

* Ground-floor room, north light, winter.

2·5 and 4·5 months at −10° C., and 9 and 9 months at −18° C. The yellow colour which develops in the superficial layers of the fat has already been discussed (page 189).

Bacon

The properties of bacon are quite different from those of pork. When pork is converted to bacon, either by the tank or dry-salt process, the meat as a whole becomes much more resistant to microbial spoilage. This is due largely to the inhibiting effect of the salts taken up by the tissues, assisted in the case of smoked bacon by a partial drying of the surface, and by the antiseptic action of traces of substances such as derivatives of catechol and formaldehyde from the wood-smoke. At temperatures above 0° C. bacon will remain unspoiled by micro-organisms for longer periods than fresh meat, and sufficiently long in many cases* to permit the development of oxidative rancidity.

Effect of Curing on Oxidation.—In addition to increasing resistance to micro-organisms, curing markedly increases the susceptibility of the fat to oxidation. The exposed fat of bacon freshly cured and matured already contains appreciable amounts (2–7 ml. per g.) of peroxide oxygen[47], and oxidises with a much less pronounced induction-period than the fat of fresh pork or beef[50]. Thus, bacon, in contradistinction to pork, becomes rancid comparatively rapidly even at −10° C., and cannot be kept by the usual methods of refrigeration for more than a month or two. In Fig. 35 are given peroxide-values, determined on very thin (0·5 mm.) superficial layers of the exposed edge of the back fat of sides of bacon stored at −10° C. for 0, 85 and 152 days, and subsequently in darkness at 15° C. for 18 days. Similar samples, taken from a side of pork stored at 0° C. for 60 days, showed only 0·25–0·30 ml. per gram[46].

When a whole side of bacon is stored, rancidity develops first at the exposed edges, and penetrates only slowly into the interior. Thus, green bacon, after storage for three months at −10° C., had a peroxide-value of about 50 ml. per g. at the exposed surface of the fat and only 2 ml. or less at a depth of one centimetre[50]. In very rancid specimens the oxidised zone is usually deeper than this. Sometimes, even after comparatively brief storage, the fat is found to be oxidised at a depth of several centimetres beneath the surface, but such cases usually appear to be due to the opening of the seam of connective tissue between the two layers of the back fat, thus permitting direct access of oxygen to the interior.

When a side, particularly one which has been stored for some time, is cut, it is frequently found that high peroxide-values and the accompanying yellow colour and rancid taste develop in certain areas (which are often adjacent to the muscle) much more rapidly

* Microbial spoilage of the fat of meat is influenced in marked degree by the humidity of the atmosphere (Fig. 32).

than in others[50]. This behaviour has also been observed in a less pronounced form in the case of pork stored at 0° C. for two months[45]

It is obvious that the process of curing reduces the resistance of pork-fat to a much greater extent than would be expected if the direct influence of temperature* on the chemical oxidation of the

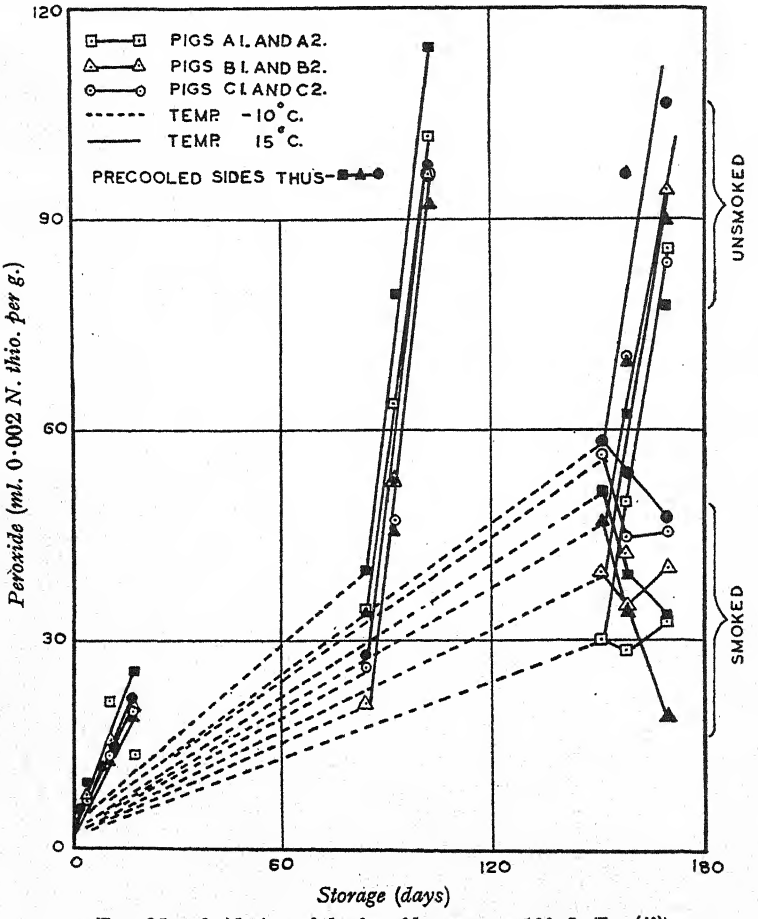

FIG. 35.—*Oxidation of the fat of bacon at* −10° *C.* (Lea[42])

fat were the sole factor involved. The effect of the curing salts has hitherto been uncertain. Sandor[70] was unable to come to any conclusion as to the effect of sodium chloride on the oxidation of lard or butter-fat, and Kilgore[43] found that salt *protected* cottonseed-

* The usual duration of the Wiltshire cure is four to five days in pickle at 5° C., followed by a further seven to ten days in a maturing cellar at the same temperature.

oil emulsified with pectin and water or made up into mayonnaise. Early experiments by Lea showed a marked accelerating effect when extracted pork-fat was stored in contact with a solution of sodium chloride in glass vessels at 25° C.[51], but later work, in demonstrating the extraordinary sensitivity of unprotected fat-water systems to copper and iron (page 157), indicated that these results were probably due to traces of metal derived from the glass and present as impurities in the salt. Sodium chloride in solutions buffered at various *p*H-values, or in solutions containing enzyme-free extracts of tissue, failed appreciably to accelerate oxidation, and it was concluded that salt has no direct effect on the rate of oxidation of lard[53, 55]. Banks[6] has also found sodium chloride to be without influence on the oxidation of herring-oil emulsified with water and calcium palmitate.

No survey of the metal contents of pickles has been made, but two examples examined contained only 0·02 parts of copper per million which, in the presence of protein, is too small to be significant. Traces of nitrite, such as are present in pickle, powerfully accelerate the oxidation of fat at *p*H-values below 5, but it is doubtful whether the acidity of the fatty tissues is ever high enough for nitrite to have much effect. It seems therefore that some other factor must be sought to explain the rapid oxidation of the fat of bacon.

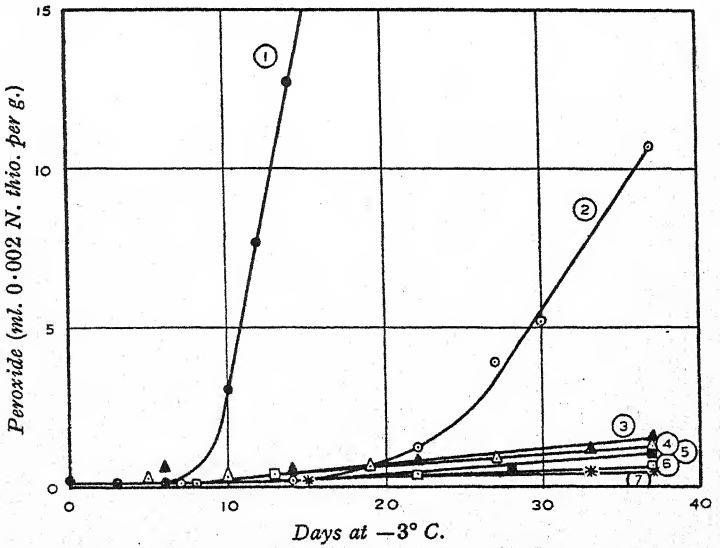

FIG. 36.—*The effect of pork-muscle lipoxidase on the oxidation of extracted pork-fat.* (Lea[52])

(1) Fat (100 g.) + muscle-juice (15 g.) + sodium chloride (1 g.).
(2) Fat + muscle-juice.
(3) Fat + heated muscle-juice + sodium chloride.
(4) Fat + heated muscle-juice.
(5) Fat + water (15 g.) + sodium chloride.
(6) Fat + water.
(7) Dry fat.

Lea in earlier papers[46, 47] suggested that the rapid, non-uniform oxidation observed in bacon, and more rarely in pork, might be due to the influence either of an enzymic system or of some other oxidative catalyst, such as (possibly) hæmoglobin. Proof has recently been obtained of the existence of such a system.

The Lipoxidase of Pork.—Various oxidising enzymes are known to be present in muscle, and an indophenol oxidase has been detected in the fatty tissues of pork[54]. Other experiments[52] have shown that pork-muscle juice contains an enzyme ("lipoxidase" or "oleinase") which accelerates the oxidation of pork-fat, an effect which can be destroyed by heating the juice, or increased by adding sodium chloride (Fig. 36). The adipose tissue itself contains a similar enzyme, and a small quantity is present in tank-pickle, presumably derived from meat previously cured in it. The existence of this enzyme and the effect of sodium chloride upon it together offer a probable explanation of the increased rate of oxidation

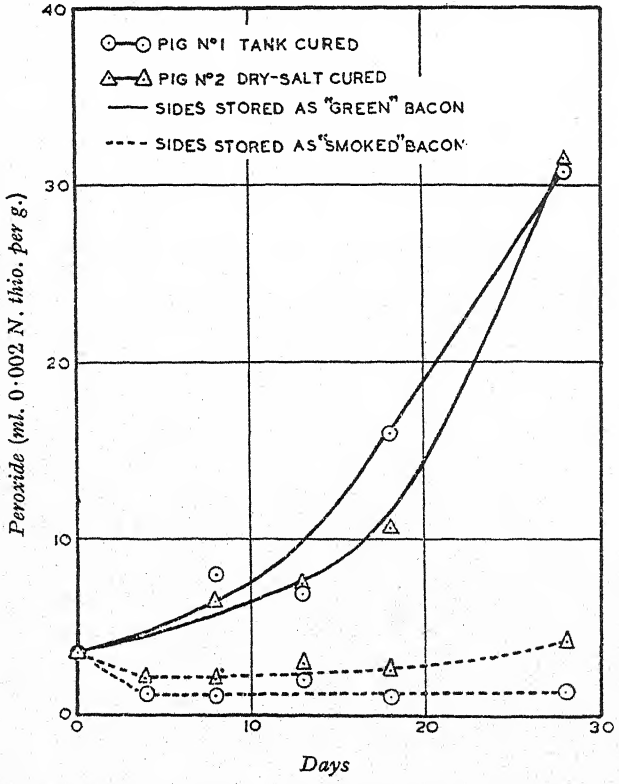

FIG. 37.—*The effect of smoking on the rate of oxidation of the superficial fat of sides of bacon during storage at* 15° *C.* (Lea[51])

observed in the fat of bacon as compared with that of pork. Preliminary results[52] indicate that the activity of muscle-juice lipoxidase is high between *p*H 4 and 5, falling with decreasing acidity to low values above *p*H 6. The enzyme is readily inactivated by heat, exposure to a temperature of 60°C. for 5 minutes being sufficient to destroy most of its activity.

Gas-storage.—Several means are available whereby oxidative spoilage in the fat of bacon can be combated. Storage at still lower temperatures undoubtedly reduces the rate of deterioration, but is expensive. Smoking the sides retards oxidation of the superficial layers of the exposed fat (Fig. 37), and even has some effect in arresting oxidation already well advanced (Fig. 35). Its usefulness is limited by the fact that, from consideration of the " bloom " or appearance of the bacon, it is usually desired to smoke *after* storage rather than before, and because on prolonged storage rancidity develops in the interior of the fat beyond the region penetrated by the smoke[45]. The efficacy of the smoking process is probably due to the absorption by the superficial layers of the fat of traces of substances, possibly phenols, which retard oxidation*. Experiments in the laboratory on the storage of bacon in atmospheres of carbon dioxide at various temperatures between 5° and —10° C. have recently been successful in inhibiting the development of rancidity for prolonged periods[15, 16], and experiments on a semi-commercial scale are now being carried out. Sides stored in carbon dioxide at 0° C. for four months were still free from rancidity, though there had been some deterioration in the texture of the lean. At —3° and —10° C. the period could be extended to eight months without spoiling the fat, and bacon stored at the latter temperature was similar in appearance and flavour to the freshly cured product. Even after 12 months at —10° C. the gas-stored bacon was still saleable, though now showing signs of incipient rancidity. Control samples in air were definitely rancid after four months. Table 50 gives the results of determinations of peroxide oxygen and flavour on 3 mm. samples taken from the exposed edge of the back fat of sides of bacon after storage at —10° C. for four, seven and 12 months in air and in carbon dioxide. In all cases the sides were smoked before examination. Similar data for earlier experiments have been given in a previous publication[15].

The usefulness of carbon dioxide for the storage of bacon on the commercial scale will probably depend on the minimal concentration of the gas necessary for the preservation of the fat, or more correctly on the maximal amount of oxygen permissible in the store. In the small-scale experiments carried out to date, the displacement of practically the whole of the air from the tanks used has been a relatively easy matter, and the atmosphere has not contained more, and may have contained considerably less, than 0·5 per cent. of

* Other possible causes are an antioxidant effect of the pea-meal used for dusting, or inactivation of lipoxidase in the superficial layers of the fat.

oxygen. Such concentrations would be difficult to attain in a large chamber. The protective effect of smaller percentages of carbon dioxide is not known. Data already quoted (page 123) indicate that during the induction-period the rate of oxidation of thin films of fat is not reduced in proportion as the concentration of oxygen in the atmosphere is decreased. On the other hand, the rate of oxidation after conclusion of the induction-period is probably much more affected. The outermost surface of the exposed fat of freshly cured bacon is already approaching, and in some cases has reached, the

TABLE 50.—*The development of oxidative rancidity in the fat of bacon stored in air and in carbon dioxide at* —10° *C.**

(Callow[17])

Type of cure	Time in store (months)	Atmosphere	Loss in weight during storage (%)	Loss in weight during smoking (%)	Flavour of the cooked back fat	Peroxide-content of the back fat (ml. 0·002 N. thio. per g.)
Dry salt	4	Air	1·9	4·3	Definitely rancid ..	14·7
„		Carbon dioxide	0·4	5·4	No sign of rancidity	4·9
„	7	Air	7·0	3·4	Definitely rancid ..	16·1
„		Carbon dioxide	1·7	5·2	No sign of rancidity	3·2
„	12	Air	9·1	2·5	Excessively rancid	32·2
„		Carbon dioxide	1·9	4·7	Very slightly rancid	3·6
Tank	4	Air	1·9	6·2	Definitely rancid ..	29·2
„		Carbon dioxide	0·0	6·2	No sign of rancidity	3·2
„	7	Air	4·7	4·1	Excessively rancid	83·7
„		Carbon dioxide	2·0	4·9	No sign of rancidity	6·5
„	12	Air	6·3	5·3	Excessively rancid	75·2
„		Carbon dioxide	0·8	5·8	Very slightly rancid	7·8

* Examination of the fat carried out by Lea.

end of its induction-period, though a rancid flavour in the fat as a whole is not detectable (particularly in smoked bacon) until oxidation in the outermost layer has proceeded beyond this point. It is to be expected therefore that storage in atmospheres containing appreciably less than 100 per cent. of carbon dioxide will have some protective effect, but experiment is necessary to ascertain how much.

Chilled Beef

Factors involved in the deterioration of frozen meat are (*a*) loss of bloom, due to desiccation of the surface and conversion of hæmo-

globin to methæmoglobin in both muscle and fat; (*b*) changes in the muscle, leading to the formation of "drip" on thawing and consequent loss in juiciness and flavour; and (*c*) oxidative rancidity and slight enzymic hydrolysis in the fat. When storage is at atmospheric or even at chilling temperatures, the case is different. Microbial spoilage of both muscle and fatty tissues proceeds so rapidly that fresh meat usually becomes unpalatable from this cause before oxidative rancidity has time to become serious. At ordinary temperatures rapid multiplication of numerous types of organisms, mainly bacteria, renders the meat inedible within a few days. Below 5° C. the bacterial flora is restricted to a few types, predominantly strains of *Achromobacter* and *Pseudomonas*, and these, together with moulds and yeasts, are largely responsible for spoilage in chilled meat. Bacteria tend to predominate when the amount of moisture available is high, as on cut surfaces of muscle with low rates of air circulation and high humidity, whereas moulds and yeasts predominate when it is low.

After storage at chilling temperatures for long periods, beef sometimes shows a considerable growth of "slime," which consists of a continuous film of colonies of *Achromobacter*, growing particularly on exposed surfaces of muscle. A little slime may occasionally be present in places where the fat covering the muscle is thin, but under normal conditions of storage, slime is rarely encountered on thick fat. Bacteria to the number of some millions per gram are, however, usually to be found in tainted fat, though the fat shows no obvious signs of their presence. Mould, and to a less extent colonies of yeasts, are more frequently visible on the surface of fatty tissues, and mould occasionally follows channels of connective tissue for considerable distances beneath the surface. In cutting up the carcase, portions of the surface showing slime or mould can be trimmed away, and the flavour of the underlying muscle is usually not seriously affected. The fatty tissues, on the other hand, may be tainted both by direct microbial attack and by the absorption of odour from adjacent slime-affected areas. The fat is therefore frequently inferior in flavour to the lean, and its deterioration is one of the most important factors in limiting the storage-life of the meat[46].

Gas-storage.—Much attention has recently been directed to the problem of increasing the storage-life of chilled beef. For many years practically the whole of the chilled beef imported into this country came from South America, being on the average 30–33 days old at marketing. Under the conditions employed it was generally estimated that the longest life of the meat was of the order of 35–40 days, so that if the ship were seriously delayed by bad weather, it sometimes became necessary to freeze the cargo. The journey from Australia and New Zealand required about 50 days, and all meat arriving from these Dominions was necessarily frozen.

Within the last three or four years increasing amounts of chilled beef have been brought from Australia and New Zealand, and the

trade is now well established. This has been made possible by the discovery at the Low Temperature Research Station, Cambridge, that 10 per cent. of carbon dioxide in the atmosphere inhibits the growth of mould and bacteria on meat, whilst at the same time it has no deleterious effect on bloom. [59, 49, 12, 83, 34, 13, 60, 61] Rigorous care in dressing the meat at the works in Australia[31] and New Zealand[10], by reducing the average bacterial load, has also helped to extend its storage-life (page 62). At present, with proper handling and with storage on board ship at a temperature of approximately −1·4° C. and in an atmosphere containing 10 per cent. of carbon dioxide, chilled beef can be kept free from mould, slime and taint for at least 70 days. The effect of carbon dioxide on the development of taint in the fat of beef at 0° C. has already been discussed (page 68).

Oxidation of the fat of chilled beef stored in air does not normally occur to any appreciable extent, and oxidative deterioration during exposure for sale is largely dependent upon the amount of light, particularly sunlight or direct sky-light, received by the meat[46]. During prolonged storage in atmospheres containing carbon dioxide, it is possible for oxidation to occur. Bleached greyish or white patches have been observed on the external fat of quarters stored both in the laboratory and on the commercial scale, usually appearing first in regions where the covering of fat is thin or where bruising has occurred. No detailed investigation of the cause of this phenomenon has yet been undertaken, but in a number of samples examined the red oxyhæmoglobin originally present had become completely converted to methæmoglobin, which is brown and a weaker pigment, and in some the yellow carotinoid pigment of the fat had been destroyed. Peroxide values of the bleached fat tended to be higher than those of adjoining fat of normal appearance, figures up to 10 ml. per g. in 3 mm. samples being observed. Fats affected in this way possessed a tallowy odour, and in more advanced cases a characteristic " oily " flavour, which latter was not, however, usually considered so objectionable as the bitter or cheesy flavour of fat tainted by micro-organisms. The patchiness of this type of oxidation, which bears some analogy to that observed in pork and bacon, suggests that an enzyme or other oxidative catalyst is involved. Such a catalyst could be derived from the tissue itself, from fluid entering from the muscle, or, less probably, from oxidase-producing yeasts or bacteria.

Fish

At atmospheric temperatures, and even in ice, microbial spoilage of fish occurs so rapidly that deterioration in flavour due to oxidative rancidity is of minor importance. At freezing temperatures microbial growth is inhibited, and in fatty fish such as herring, salmon, smelt, etc., oxidative rancidity readily develops, together with the discolouration and resinification known as " rusting," which has already been discussed (page 189).

Owing to the highly unsaturated nature of fish-oils, and probably to the presence of enzymes which accelerate the process, oxidation proceeds with considerable rapidity under ordinary conditions of cold storage and very low temperatures are necessary for successful storage for long periods. Taylor[80] states that low temperature is the only sure means of avoiding rust or rancidity, though a heavy glaze is of great benefit. Tressler[85] recommends temperatures of −28·9° C. or lower. Small unglazed fish (smelt) held in a chamber at −15° C. have been found to oxidise so rapidly as to maintain their surface above the lower limit of temperature for the growth of mould (−7° C.)

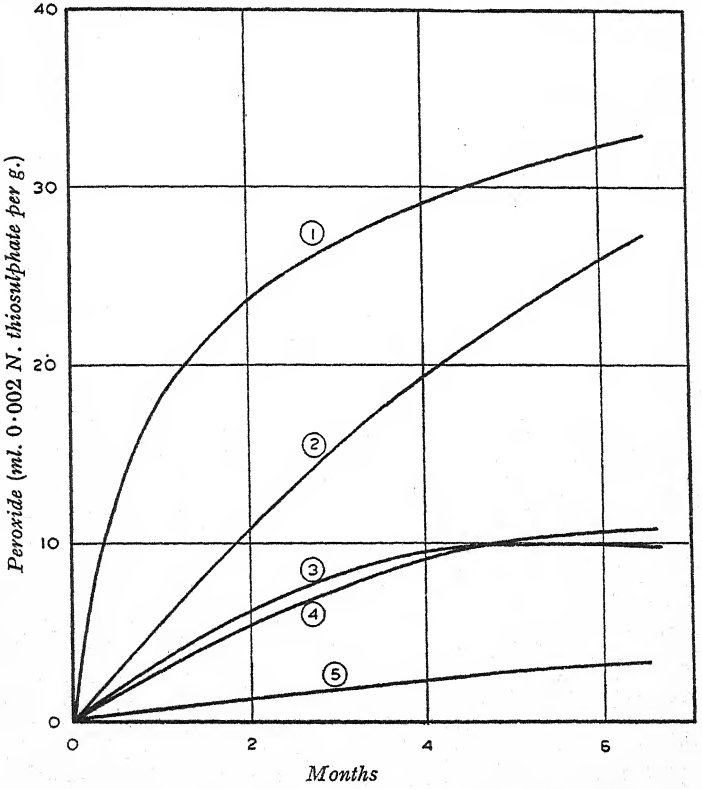

FIG. 38.—*Oxidation of the superficial* (1 *mm.*) *fat of cold-stored herring.* (Banks[7])

(1) Brine-frozen, unglazed, −20° C.
(2) „ glazed, −20° C.
(3) „ „ −28° C.
(4) Air-frozen, unglazed, −20° C.
(5) „ glazed, −20° C.

Estimation of peroxide and determination of free acidity on the extracted oil have been used by Stansby[75] as means for following oxidative rancidity and microbial spoilage in mackerel. In case of the

herring, Banks (Fig. 38) has come to the conclusion that (1) brine-frozen fish become rancid more rapidly (three months) than air-frozen controls, which in these experiments were still free from rancidity after six months at −20° C.; (2) protecting the fish by a glaze of ice improves their keeping properties; and (3) low temperatures of storage greatly delay the appearance of rancidity.

TABLE 51. *Effect of muscle on the oxidation of herring-oil at* −10° *C.* (Banks[6])

Days	0	10	17	28	40	56	63	71
Herring-muscle + herring-oil	3·6*	9·3	11·5	21·0	22·5	34·5	67·0	132·6
Herring-muscle (sterilised) + herring-oil ..	3·6	7·7	10·2	12·7	14·1	22·6	39·6	30·7
Herring-muscle + herring-oil + sodium chloride	3·6	10·1	12·8	20·7	25·8	63·9	157·8	270·6
Herring-muscle (sterilised) + herring-oil + sodium chloride ..	3·6	8·2	10·3	14·9	20·3	41·5	44·6	37·6
Blank experiment ..	3·6	7·2	6·3	7·2	7·7	11·7	11·4	16·3

* Ml. 0·002 N. thiosulphate per g. of fat.

In subsequent work[6] minced herring's muscle was found to catalyse the oxidation of herring-oil, an effect which was increased by sodium chloride and destroyed by heat (Table 51). This action is considered to be due to an enzymic system present in the muscle.

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